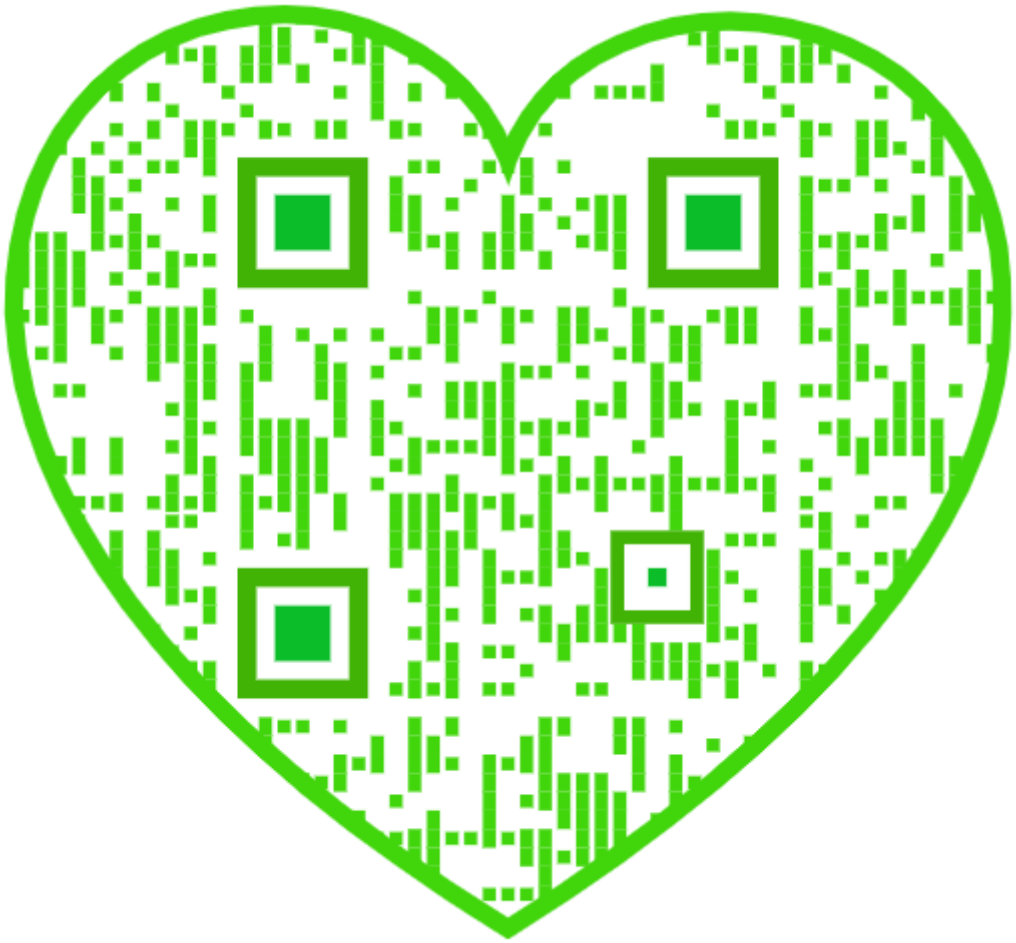

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ASSISTED BY OTHER BOTANISTS

VOLUME XXI

With One Portrait, Forty-six Plates, Ten Diagrams, and Thirty-one Figures in the Text

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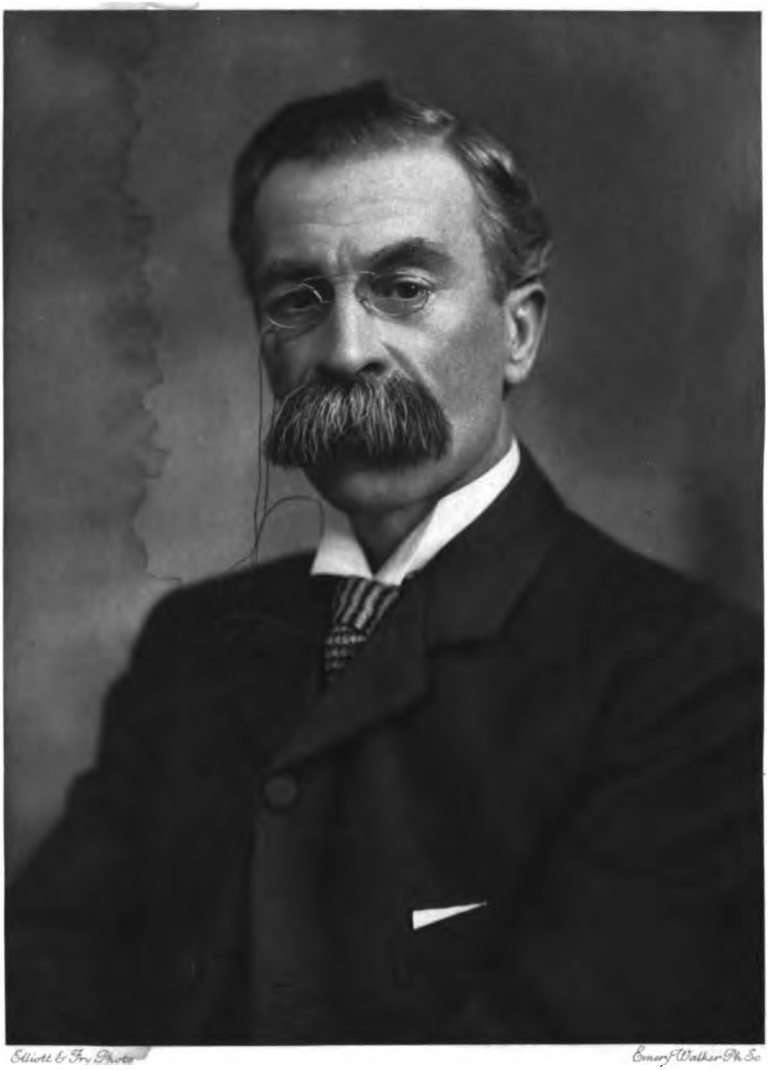

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H. Marshall Ward

HARRY MARSHALL WARD.

(With Portrait.)

ALTHOUGH several notices of his life and appreciations of his work have already appeared, some further account of Ward, in the pages of the Annals, if only as a tribute of regard and regret, will not be superfluous, inasmuch as he was closely associated with the inception and conduct of this periodical.

It will not, however, be necessary to dwell here upon the details of his life: it will suffice to mention the main facts. He was born at Hereford in 1854, and his scientific life began about twenty years later, in 1875, when he attended a course of practical instruction in Botany conducted at South Kensington by Sir W. Thiselton-Dyer. So deep was the impression made by his ability and enthusiasm upon his teachers, that they urged him, if possible, to enter upon a botanical career. The financial difficulties having been fortunately overcome, partly by a scholarship at Christ's College, Cambridge, Ward went into residence at the University in October, 1876. As an undergraduate he fully availed himself of the opportunities of study in all branches of Biology, gaining thereby a breadth of knowledge and of outlook that stood him in good stead in his subsequent work, and secured for him a first class in the Natural Science Tripos of 1879.

No sooner had he taken his degree than he threw himself into research with characteristic ardour, his first published papers (see appended list) being dated 1880. At the same time he prosecuted his botanical studies under Professor Sachs at Würzburg and Professor de Bary at Strassburg; and it was doubtless from the latter that he received the impulse which led him to devote himself especially to Mycology. But these studies were cut short by his appointment by the Government of Ceylon as cryptogamic botanist to investigate the Coffee-Leaf Disease that was ravaging that island. He spent two years there, and, though he successfully elucidated the life-history of the Fungus, he was unable to discover any effectual remedy. On his return to England he was elected a Berkeley Fellow at Owens College, Manchester, and became assistant to the late Professor Williamson, F.R.S. In 1883 he was elected a Fellow of Christ's College,

Cambridge, and in 1885 he left Manchester to occupy the Chair of Botany in the Forestry Department of the Royal Indian Engineering College, Cooper's Hill. There he remained for ten years, and though closely occupied with his official duties he found time to carry out several of his most important researches, especially those upon the bacteriology of the Thames. Moreover his routine work led him to enter upon a new line of study, that of timber and trees, of which the outcome is to be found in his books on the Oak, on Timber, on Trees, &c., as well as in the works, such as his paper on *Stereum* and his books on plant-diseases, which include also mycological research. In fact Ward, at this stage, seems to have become profoundly impressed with the desirability of combining as far as possible scientific research with the attainment of practical ends, an attitude that found its fullest expression in his presidential address at Toronto in 1897, which dealt with the economic significance of the Fungi.

In 1895 Ward was called to succeed Professor Babington in the Chair of Botany at Cambridge, and here, perhaps for the first time, he found himself in a wholly congenial atmosphere with full and free scope for all his activities. Under his care the botanical school flourished, and so increased in numbers and importance that the University erected for its accommodation a large and well-equipped institute which, together with other buildings, was opened by His Majesty the King in March, 1904.

It was during this period that he pursued his last and perhaps most important line of research, the investigation of the Rusts that infest the Brome-grasses. He established the existence of physiological races of these fungi, showing that certain species of Brome can only be infected by certain breeds of Rusts; and, from the point of view of his Croonian lecture of 1890, he endeavoured to ascertain the causes of immunity and of infectibility. Without having absolutely solved the problem, he proved that the structure of the host is not the determining factor, and made it probable that this factor is to be sought in the secretion of enzymes or toxins by the would-be parasite on the one hand, and of ant-enzymes and anti-toxins by the host on the other. Incidentally he had occasion to investigate the 'Mycoplasm' theory of Professor Eriksson, and failed to confirm his observations. This led to a spirited discussion of the subject on the occasion of the meeting of the British Association at Cambridge in 1904.

Soon after this it became noticeable that Ward's health was giving way. He continued his professorial work as far as his strength permitted, but his increasing weakness was only too evident. The end came, somewhat unexpectedly, during a stay at Torquay, on Sunday, Aug. 26, 1906; and on Sept. 3 he was laid to rest in the Huntingdon Road Cemetery, Cambridge.

His merits did not pass without recognition during his life. In 1886

he became a Fellow of the Linnean Society of London, and was elected to the Royal Society in 1888, receiving a Royal Medal in 1893; he served on the Council of the Linnean Society 1887–9, and on that of the Royal Society 1895–6. He was elected an Honorary Fellow of Christ's College, Cambridge, in 1897, and received the degree of D.Sc. *honoris causa* from the Victoria University in 1902. He was President of the British Mycological Society 1900–2, and had received the honorary fellowship of various learned societies.

And now that he is gone from us, we, his old friends and colleagues, would honour his memory by expressing, however inadequately, our deep sense of the loss we have sustained. We recall his unswerving loyalty and effective co-operation as a fellow-pioneer in this country of what, a quarter of a century ago, was derisively termed the 'New Botany,' though it was but a renascence of the botany of Hales, Knight, Robert Brown, and Henfrey: we remember his varied gifts, his geniality, his untiring industry, his never-failing enthusiasm: we recognize what he has achieved for the study of Mycology in Britain, carrying on the work of Berkeley and maintaining his high standard. Above all, we are conscious that the gap left in our ranks can never be filled for us. But we draw comfort from the confidence that the future of the cause for which both he and we have striven is assured: new ranks are forming, recruited largely from among Ward's pupils, ready to take his place, and ours, in the fighting-line. To these Ward's life and work may well serve as an inspiration to enthusiasm and an ideal of devotion.

S. H. V.

LIST OF PAPERS.

1880. On the Embryo-sac and Development of *Gymnadenia conopsea*. Quart. Journ. Micr. Sci., vol. xx.

A Contribution to our Knowledge of the Embryo-sac in Angiosperms. Journ. Linn. Soc. Bot., vol. xvii.

1880–1. Three Reports on the Coffee-Leaf Disease, published by the Government of Ceylon.

1882. Researches on the Life-History of *Hemileia vastatrix*, the Fungus of the 'Coffee-Leaf Disease.' Journ. Linn. Soc. Bot., vol. xix.

1883. On the Morphology and the Development of *Meliola*, a Genus of Tropical Epiphyllous Fungi. Phil. Trans. R. S., Part II.

Observations on Saprolegniae. Quart. Journ. Micr. Sci., vol. xxiii.

Observations on the Genus *Pythium*. Ibid.

1884. On the Structure, Development, and Life-History of a Tropical Epiphyllous Lichen (*Strigula complanata*, Fée). Trans. Linn. Soc., vol. ii.

On the Sexuality of the Fungi. Quart. Journ. Micr. Sci., vol. xxiv.

1887. On the Structure and Life-History of *Entyloma Ranunculi*. Phil. Trans. R.S., vol. clxxviii, B.

On the Tubercular Swellings on the Roots of *Vicia Faba*. Ibid.

1887–8. On some points in the Histology and Physiology of the Fruits and Seeds of *Rhamnus* (with Mr. Dunlop). Ann. Bot., vol. i.

Some recent Publications bearing on the question of the Sources of Nitrogen in Plants. Ibid.

1888–9. Illustrations of the Structure and Life-History of *Puccinia Graminis*, the Fungus causing the 'Rust' of Wheat. Ann. Bot., vol. ii.

A Lily-disease (*Botrytis*). Ibid.

1890. On some Relations between Host and Parasite in certain Epidemic Diseases of Plants. (Croonian Lecture) Proc. Roy. Soc., vol. xlvii (No. 290), p. 393.

On *Craterostigma pumilum* (with Miss Dale). Trans. Linn. Soc., vol. v.

1892. The Ginger-beer Plant, and the Organisms composing it. Phil. Trans. R. S., vol. clxxxiii, B.

On the Characters, or Marks, employed for classifying the Schizomycetes. Ann. Bot., vol. vi.

1892–7. Reports to the Water Research Committee of the Royal Society:

I. On the present State of our Knowledge concerning the Bacteriology of Water, with special reference to the Vitality of Pathogenic Schizomycetes in Water (with Professor Percy F. Frankland, F.R.S.). Proc. Roy. Soc., vol. li (No. 310), p. 183, 1892.

II. The Vitality and Virulence of *Bacillus Anthracis* and its Spores in Potable Waters (with Professor Frankland).
Experimental Investigations on the Behaviour of *Bacillus Anthracis* in Water (with Dr. Cartwright Wood). Proc. Roy. Soc., vol. liii (No. 323), p. 164, 1893.

III. Further Experiments on the Action of Light on *Bacillus Anthracis*, and on the Bacteria of the Thames. Proc. Roy. Soc., vol. lvi (No. 338), p. 315, 1894.

IV. On the Biology of *Bacillus ramosus*, a Schizomycete of the River Thames. Proc. Roy. Soc., vol. lviii, 1895.

V. Summary. Proc. Roy. Soc., vol. lxi (No. 376), p. 415, 1897.

1893. Experiments on the Action of Light on *Bacillus Anthracis*. Proc. Roy. Soc., vol. lii (No. 318), p. 393.

1895. The Action of Light on Bacteria. Phil. Trans. R. S., vol. clxxxv, B.

The Formation of Bacterial Colonies. Ann. Bot., vol. ix.

A False Bacterium. Ibid.

1897. Presidential Address to Section K, British Association, Toronto meeting.

On *Peziza aurantia*. Ann. Bot., vol. xi.

On the Ginger-beer Plant. Ibid.

Microscopic Gardening. Journ. Roy. Hort. Soc., vol. xxi.

1898. A Violet Bacillus from the Thames. Ann. Bot., vol. xii.

Some Thames Bacteria. Ibid.

A Potato-Disease. Ibid.

Penicillium as a Wood-destroying Fungus. Ann. Bot., vol. xii.

On the Biology of *Stereum hirsutum*. Phil. Trans. R. S., vol. clxxxix.

1899. *Onygena equina*, a Horn-destroying Fungus. Phil. Trans. R. S., vol. cxci.

Thames Bacteria, III. Ann. Bot., vol. xiii.

Symbiosis. Ibid.

Some Methods for Use in the Culture of Algae. Ibid.

1901. The Bromes and their Rust-Fungus (*Puccinia dispersa*). Ann. Bot., vol. xv.

1902. On the Question of 'Predisposition' and 'Immunity' in Plants. Proc. Camb. Phil. Soc., vol. xi, p. 307.

On Pure Cultures of a Uredine, *Puccinia dispersa*. Proc. Roy. Soc., vol. lxix (No. 458), p. 451.

Experiments on the Effect of Mineral Starvation on the Parasitism of the Uredine Fungus, *Puccinia dispersa*, on Species of Bromes. Ibid., vol. lxxi (No. 469), p. 138.

On the Relations between Host and Parasite in the Bromes and their Brown Rust, *Puccinia dispersa*. Ann. Bot., vol. xvi.

1903. On the Histology of *Uredo dispersa*, and the 'Mycoplasm' Hypothesis. Phil. Trans. R. S., vol. cxcvi, B.

Further Observations on the Brown Rust of the Bromes and its Adaptive Parasitism. Annales Mycologici, vol. i.

1905. Recent Researches on the Parasitism of Fungi. Ann. Bot., vol. xix.

Books.

1884. English edition of Sachs' 'Vorlesungen über Pflanzenphysiologie.'

1889. Timber and some of its Diseases.

1892. The Oak.

1894. Edition of Laslett's 'Timber and Timber-trees.'

1896. Diseases of Plants.

1901. Grasses.

Disease in Plants.

1904–5. Trees: a Handbook of Forest Botany, vols. i–iii (incomplete).

Obituary Notices.

Nature, vol. lxxiv, 1906 (Vines).

Christ's College Magazine, vol. xxi, 1906 (Gwynne-Vaughan).

Botanisches Centralblatt, Bd. cii, 1906 (Vines).

Kew Bulletin, 1906 (Boodle).

Journal of Botany, vol. xliv, 1906 (Bower).

Gardener's Chronicle, vol. xl, 1906.

New Phytologist, vol. vi, 1907 (Thiselton-Dyer).

Proceedings of the Linnean Society, 1906–7 (Daydon Jackson).

Memoirs and Proceedings of the Manchester Literary and Philosophical Society, vol. li, 1906–7 (Weiss).

The Gametophytes, Fertilization and Embryo of Cephalotaxus drupacea.

BY

ANSTRUTHER A. LAWSON, PH.D.

Assistant Professor of Botany in Stanford University, California, U.S.A.

With Plates I–IV.

INTRODUCTION.

TO the morphologist or to the cytologist there are few groups of plants which offer more attractive features than the Coniferales. This is not only true of the sporophyte structures, but is particularly true of the gametophyte, for here every phase in the history of the sexual generation reveals the development of structures which are of profound interest in regard to their morphological meaning. The facts concerning the behaviour and development of such gametophytic structures are, therefore, from the point of morphology alone, worthy of record.

The results of investigations on the comparative anatomy of the sporophyte, together with the evidence which is gradually accumulating from the study of fossil forms, is bringing us nearer to an understanding of the true phylogeny of the Coniferales, but at present such an understanding is far from perfect. That we are not yet warranted in establishing the true phylogenetic relationships of the Coniferales and their various sub-groups, is due mainly to the fact that the strongest kind of evidence, that of the fossils, is at present disconnected and in places contradictory. In view of the gaps in the chain of evidence from the paleobotanical side, a knowledge of the primitive structures associated with the gametophyte generation becomes imperative. That our knowledge of such structures has had much to do in modifying our ideas as to the phylogeny of the various groups of Gymnosperms, we need only refer to the effect of the discoveries of Hofmeister, Strasburger, Hirasé, Ikeno, and Webber, to say nothing of the great amount of literature which has more recently accumulated on the gametophytes of the Abietineae, Cupressineae, Taxodieae, and Taxeae. Indeed, many of the discoveries recently made are so extraordinary that

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a detailed history of the gametophytes of all living forms seems quite necessary if we hope to build up a phylogeny of the Coniferales and their various sub-groups.

The present investigation is intended as a contribution to our knowledge of the Taxeae. It was undertaken with the idea of giving a complete account of the development of the gametophytes and embryonal sporophyte of *Cephalotaxus*. It thus fills in many of the gaps left by Sokolowa ('91), Strasburger ('79), and Arnoldi ('00), who have contributed fragmentary but valuable information concerning the gametophytes of this interesting genus. The work was commenced in the spring of 1903 but was delayed in its completion on account of the scarcity of material. The greater part of the work was carried on in the Botanical Laboratory of Stanford University, California, but it was finished in the Jodrell Laboratory at Kew Gardens.

I take this opportunity of expressing my gratitude to Lieut.-Col. Prain, C.I.E., F.R.S., Director of the Royal Gardens, Kew, and to Dr. D. H. Scott, F.R.S., Honorary Keeper of the Jodrell Laboratory, who kindly extended to me the privileges of the Jodrell Laboratory, and thus made the completion of the work possible. To Mr. L. A. Boodle, F.L.S., also I wish to express my sincere thanks. Upon my arrival at Kew Gardens I found that Mr. Boodle had commenced an investigation upon the gametophytes of *Cephalotaxus Fortunei* and *C. drupacea*, and had collected and prepared considerable material for this purpose. Upon learning that my investigation was so near completion, both as regards drawings and text, Mr. Boodle kindly invited me to compare some of his excellent preparations with my own. The result of this comparison was very gratifying, for it helped to confirm several important interpretations which might otherwise have remained in doubt.

METHODS.

The material for this investigation was obtained from three small shrubs growing on the campus of Stanford University, California. I began to make collections early in March, 1903, and continued to prepare material at short intervals, as long as time and the supply of material permitted, until the spring of the present year. There was not an abundance of fruit formed on the shrubs and I therefore thought it best to sacrifice the early stages of the female gametophyte in order to have enough specimens for investigating the later phases. I hope in the near future to trace out the formation of the megaspores and related structures.

For those who are investigating the cytology of the Gymnosperms I should like to emphasize the importance of fixing the material in the field or, when that is impossible, to otherwise provide against evaporation. With such critical stages as the division of the body-cell, the organization

of the ventral canal-cell, and fertilization, a rapid fixation is quite necessary. Better results were always obtained when the material was placed in the killing fluid as soon as possible after being detached from the tree. For many stages it is even desirable to carry on the dissections while the material is submerged in the fixing fluid.

The ordinary cytological methods were employed, details of which have already been published (Lawson, '04). We need only add that Flemming's weak solution and the chrom-acetic mixture gave the most satisfactory results as fixing reagents. The triple combination, safranin, gentian violet, and orange G. was used for staining.

The Male Gametophyte.

The first collection of the microsporangia was made on March 1, before the megasporangia were visible. From then on to the time of pollination collections were made almost daily. The microspores from the early collections contained a single large central nucleus and a large quantity of starch grains. The spore at this time also showed a well-developed exine. Fig. 1 represents a cross-section of a young microspore just before its first division. Sections of the sporangia taken a day or two before the pollen was shed showed two distinct nuclei in each microspore, although I was unable to find the spindle showing the actual division of the first nucleus. One of these nuclei was larger than the other and more centrally located. The former is undoubtedly the tube nucleus, while the history of the smaller one proved it to be the generative nucleus. From the observations of Strasburger ('92) and Coker ('04) it appears that in *Taxus*, *Juniperus*, and *Cupressus* there is no division of the microspores during their confinement within the sporangium, while in the Abietineae and in *Chamaecapyris*, *Cryptomeria*, *Thuja*, *Sequoia*, *Podocarpus*, and *Cephalotaxus* the microspores divide before they are shed.

In *Cephalotaxus drupacea* the first division of the microspore takes place at least a day or two before pollination. Fig. 2 represents a section of a pollen-grain at this time. It will be observed that the starch grains, which were formerly present in abundance, have entirely disappeared, and that there is an indication of a delicate membrane separating the two nuclei. In view of the importance of these early stages in the history of the male gametophyte, and especially in view of the fact that *Cephalotaxus* is regarded by many botanists as a primitive type of Conifer, a very careful search was made for any evidence of the survival of sterile prothallial cells as found in the Cycads, *Ginkgo*, and in the Abietineae, but no trace of such vestigial structures were found. There were but two nuclei present, and a careful study has convinced me that the division which gives rise to them (i. e. the generative and tube nuclei) is the only division in the male gametophyte which precedes the organization of the body and stalk

nuclei. A similar condition has been reported for *Taxus* (Coker, '04) and *Torreya* (Robertson, '04; Coulter and Land, '05). Indeed, from the number of types that have been investigated, it would seem that the survival of the sterile prothallial cells among the Coniferales, is almost entirely confined to the Abietineae. The single exception is that of *Podocarpus*, and even here there are strong suspicions that the Podocarpeae and Abietineae are closely related (Coker, '02).

In this connexion it is interesting to note that Miss Robertson ('04)[1] correlates the survival of the vegetative prothallial cells with the winged character of the pollen-grain. No one doubts the primitive character of the Cycads and *Ginkgo*, and here the prothallial cells are evident. They are also constantly present in the Abietineae, but in the Taxeae, Taxodineae, and Cupressineae they are conspicuous by their absence. Miss Robertson ('04) infers that the superior buoyancy produced by the bladder-like swellings of the exine in *Podocarpus* and in the Abietineae 'would make any difference in weight of very little importance, and so natural selection would not come into play to eliminate the vestigial sterile cells in the pollen-grain.' In her explanation Miss Robertson assumes that the elimination of the one or two prothallial cells within the pollen-grain was accomplished in order to bring about a reduction in its specific gravity; and also that it was easier for natural selection to develop new structures in the form of winged bladders than to eliminate one or two very minute cells which have long since become functionless. Personally, I question whether the presence of one or two very minute prothallial cells within the pollen-grain would make any perceptible difference in its specific gravity. The early reduction in the number and size of the cells in the prothallium was no doubt accomplished to meet this end, thus affording an easy transportation; but I am more inclined to believe that the final elimination of the last two functionless vestigial cells in the Taxodineae, Cupressineae, and Taxineae was the result of long disuse. My belief in this regard is strengthened by a fact which Miss Robertson has evidently overlooked. It is well known that in the Pine pollen the vestigial prothallial cells become disorganized very soon after they are formed, and practically vanish *before* the pollen is discharged from the sporangium. This is also true for other members of the Abietineae. Miyake ('03) reports that in the pollen-grain of *Picea*, just before pollination, 'the disintegrating remains of the first two prothallial cells can be seen merely as two thin and darkly staining bodies between the stalk-cell and the pollen wall.' With the practical disappearance of the prothallial cells in the winged pollen, *before* the bladder-like appendages have an opportunity of producing any buoyancy, it is difficult to understand how these latter structures can be responsible for the survival of the former.

[1] See also Jeffrey, E. C., The Comparative Anatomy and Phylogeny of the Coniferales, Part II. Mem. Bost. Soc. Nat. Hist., 1904.

During the time that the pollen is free, there is practically no difference in the cellular contents of the grain of the winged pollen of the Abietineae and the wingless pollen of the majority of other Conifers. It thus seems to me that the correlation of the winged character of the pollen and the survival of the prothallial cells is not quite consistent. To my mind the survival of these latter structures is an indication of the primitive character of the Abietineae as a group.

The discharge of the pollen in *Cephalotaxus* begins late in March and continues for about three weeks. A considerable difference in the time of pollination was noted from year to year, which was probably due to the variable seasons in California. When the pollen is shed, the megasporangium is but a very small pointed protuberance surrounded by a well-developed integument. The micropyle is a little longer than the sporangium, as shown in Fig. 3, and remains open for some time after pollination. The microspores become lodged on the top of the megasporangium at the base of the micropyle, and here they remain; and although they enlarge considerably, there is not much further germination until the following spring. Early in May of the following year the pollen-tube becomes visible and begins its downward penetration into the tissue of the nucellus. In some cases there was observed a slight indication of the branching of the tube at this time, as shown in Fig. 4, but the main growth was always in a downward direction, directly toward the female prothallium. Previous to the penetration of the tube, the division of the generative nucleus takes place, for, as shown in Fig. 4, the body-cell is already organized, as well as the stalk-nucleus. The young tube now contains one large cell and two free nuclei, and in this respect conforms with the conditions met with at this time in most Conifers. The body-cell is distinctly oval in shape, and is surrounded by a distinct membrane. It contains a dense granular cytoplasm and a large, deeply-staining nucleus, which is quite four or five times the size of the stalk or tube-nucleus. One constant character of the body-cell is that its long axis is parallel to the long axis of the tube, and that its large nucleus is invariably found near the cell-membrane, at the side away from the stalk and tube-nuclei, as shown in Figs. 4 and 5. The stalk and tube-nuclei show no perceptible increase in size as the tube advances. They are almost identical. Indeed, it is quite impossible, at this time, to distinguish the one from the other. They lie close together, imbedded in a mass of cytoplasm close to and always in advance of the body-cell.

From this time on, the growth of the tube is comparatively rapid. The condition of the tube and its contents, shown in Fig. 4, was found on May 11, while that in Fig. 5 was found on May 21, taking just ten days for the tube to penetrate completely through the nucellar tissue and reach the archegonial chambers at the upper part of the female prothallium. The nucellar tissue, through which the tubes push their way, has a lacerated appearance,

and the cells near the tubes become thoroughly disorganized. The number of tubes varied from one to four, but more generally only one or two were found in a single nucellus. As the tube advances, the body-cell and the tube and stalk-nuclei were invariably situated near the tip, as shown in Fig. 5. When the tip of the tube has penetrated through the nucellar tissue and has reached the space between the nucellus and the archegonial chambers, it becomes quite distended, as if a great osmotic pressure were acting from within. It would seem that the sudden release from the confinement of the surrounding nucellar tissue permitted the wall of the tip of the tube to stretch, allowing the latter to take on an almost spherical or bulb-shaped form, as represented in Figs. 5 and 6. It will also be seen from these figures that the bulk of the cytoplasm is at the tip of the tube, with a thin layer lining the wall.

As soon as the tube reaches this condition the nucleus of the body-cell enlarges and prepares for division. This stage is shown in Fig. 6. I was unable to find the spindle of the dividing body-nucleus, but a careful study of the cytoplasm before and after the spindle stage revealed nothing that would indicate the presence of blepharoplasts, which occur during the division of the body-cell in the Cycads (Ikeno, '98; Webber, '97) and *Ginkgo* (Hirase, '95). The cytoplasm immediately before division was finely granular at the periphery, but distinctly fibrilar in its structure in the vicinity of the nucleus, which indicated the preliminary stages in the formation of the spindle.

The result of the division of the body-nucleus proved very interesting because it differed in a marked degree from that reported for *Taxus* (Belajeff, '93) and *Torreya taxifolia* (Coulter and Land, '05). It will be remembered that in *Taxus* this division results in the organization of two male cells of unequal size. In *Torreya taxifolia* a similar condition prevails, although Miss Robertson ('04) reports an equal division of the body nucleus in *Torreya californica*, and thus similar to the conditions found in *Cephalotaxus Fortunei* by Arnoldi ('00). Miss Robertson, however, believes that only one of the male nuclei enters the archegonium. The discovery of Juel ('04) that the body-cell in *Cupressus Goweniana* gives rise to a complex of cells is an interesting and important one, especially as Thomson ('05) and Lopriore ('05) recently report that in *Agathis australis* and in *Araucaria* the pollen-tube may contain six or seven nuclei. In these latter cases it will be interesting to learn whether these nuclei are vegetative or generative.

In *Cephalotaxus drupacea* I was able to follow a very complete series of stages after the division of the body-cell nucleus, and my observations are these:—There is no cell-plate formed between the two male nuclei, and consequently there are not two male *cells* formed, but simply two large nuclei, which lie within the old membrane of the body-cell. These nuclei

are at first perfectly spherical and of equal size. As shown in Figs. 7 and 8 they eventually lie close together and soon lose their spherical form. They become slightly elongated and seem to cling or fold about each other, as shown in Fig. 8. This condition was very frequently observed just prior to their entrance into the archegonium. When the sperm-nuclei are completely organized the chromatin is in the form of uniformly small granules with two or three deeply-staining nucleoli.

Surrounding the nuclei there is very little starch present, which is in contrast to the conditions generally found at this stage in the Cupressineae, and to which Coker ('03) seems to attach some importance.

As we shall point out later, both sperm-nuclei, still folding over one another, may enter a single archegonium together, or they may separate a short distance apart just before entering. It invariably happens, however, that they both enter the same archegonium.

The history of the male gametophyte in *Cephalotaxus drupacea* covers a period of about fifteen months. In none of its phases does it show any noticeably primitive characters as compared with other Coniferales.

The Female Gametophyte.

There were two difficulties in the way which prevented the securing of a satisfactory series of preparations to show the development of the megaspores. First, because the three trees, from which all the material was obtained, did not produce flowers in abundance, I hesitated to sacrifice the later history of the gametophyte for the sake of the early megaspore stages; and second, because the hard thick tissue enveloping the young ovule prevented the rapid penetration of the killing fluid, it was almost impossible to obtain a satisfactory fixation of the cells concerned, the ovules themselves being too small to remove without injury. In one preparation I was able to make out a single large megaspore mother-cell and in another a large megaspore with two smaller cells, one above the other, which I interpret as disorganizing megaspores. As to the reduction division and the events immediately following I was unable to trace further.

It seems almost certain, however, that only one megaspore germinates, and that its early germination proceeds very slowly. As the megaspore enlarges, the usual free nuclear division takes place. In several cases six or eight free nuclei were found in the cytoplasm. A central vacuole very soon makes its appearance, which increases in size and gradually forces the cytoplasm and free nuclei to the periphery. Fig. 9 shows a longitudinal section through the young female prothallium, with the very large central vacuole and the parietal layer of cytoplasm, in which the free nuclei are distributed at more or less regular intervals. Up to this time the parietal layer of cytoplasm is but a very delicate film. As shown in Fig. 12 it is not even as wide as the diameter of the nucleus. The character of the

parietal layer was very similar to that which I have studied in *Sequoia* and *Cryptomeria.* As indicated in Fig. 10, the central vacuole increases enormously without much perceptible difference in the thickness of the parietal layer of cytoplasm, although there is a very noticeable increase in the number of nuclei, which shows that free nuclear division proceeds during the enlargement of the vacuole.

During these early stages in the formation of the prothallium considerable attention was given to the nucellar tissue which immediately surrounds it. In *Taxodium* Coker ('03) describes a distinct layer of large cells which surrounds, and persists throughout the growth of the young prothallium. It is thought that this layer takes an active part in the nourishment of the growing gametophyte, and is, therefore, considered to be a tapetum. A similar more or less differentiated tapetum has been found during the early stages in the development of the prothallium in the Abietineae and the Cupressineae, but seems to be entirely wanting in *Cephalotaxus.* It is also absent in *Taxus* and *Torreya.*

For *Cephalotaxus Fortunei* Mlle Sokolowa ('91) figures a distinct but very thin megaspore-membrane. In *C. drupacea* I was unable to detect the presence of a megaspore-membrane about the young prothallium, but during the fertilization period and early embryo stages an extremely thin membrane was observed. In two other species of *Cephalotaxus* Thomson ('05) was unable to find a trace of this membrane. He also reports that in *Taxus* the megaspore-membrane is very poorly developed. The absence of the tapetum and the extreme evanescent condition of the megaspore membrane is significant. Indeed, from this point of view alone Thomson ('05) regards the Taxeae as the most recent group of the Coniferales.

After the stage shown in Fig. 10 the parietal layer of cytoplasm increases in thickness, but the nuclei remain on the inner surface near the vacuole. The formation of the first cell-walls is not unlike that found in other Conifers. The nuclei, instead of dividing freely as heretofore, have walls formed between them, thus forming the primary prothallial cells or 'alveoli' of Mlle Sokolowa ('91). These primary cells are open on the inner side and exposed to the sap of the central vacuole. The primary cells elongate rapidly and encroach upon the vacuole. Before the complete closure of the central vacuole by the inward growth of the prothallial cells numerous oblique and cross-walls are formed. These early cross-walls are also present in *C. Fortunei* (Sokolowa, '91) and *Torreya* (Robertson, '04). Fig. 11 represents a section of the upper half of the prothallium before the ingrowing cells have met in the central region. It will be noticed that the nuclei are not only distributed on the inner exposed surface but they are quite numerous at the periphery of the prothallium. The cytoplasm also seems to be concentrated at these two definite regions. Nothing unusual was observed in the final formation of permanent prothallial tissue. From

Fig. 13 it will be seen that the cells are arranged in rows which converge towards the centre of the prothallium. By comparing this with Fig. 11 one may safely infer that these slanting rows or lines of cells were formed from the ingrowing primary cells.

The Archegonia.

The first indications of archegonial initials were observed in material collected early in April, somewhat over a year from the time the megasporangium first made its appearance. They are generally but four in number and are clearly of superficial origin. In the earliest stages observed the initial cells were not much larger than the surrounding sterile cells, and only their granular cytoplasm and deeply-staining nuclei differentiated them from the latter. At a very early stage the initial divides, giving rise to the first neck-cell and the central cell. The primary neck-cell almost immediately divides by an anticlinal wall, and thus forms the two neck-cells. It frequently happens that one of these divides again anticlinally, so that the mature archegonium may present two or three cells in the neck in cross-section, as shown in Fig. 15. The neck-cells are easily distinguished from the other superficial cells of the prothallium by their densely granular contents. Among the Coniferales the number of neck-cells to the archegonium seems to vary considerably; even in the same species the number is not at all constant. There are never less than two, however, and that seemed to be the prevalent number in *Cephalotaxus*. Fig. 14 shows a longitudinal section of a young archegonium and the position of the two neck-cells from this view.

As soon as the neck-cells became organized, the central cell grows very rapidly, and its nucleus, which at this early stage is centrally located, increases to fully five or six times its original size. Its cytoplasm also becomes much more densely granular and stains more deeply. The four archegonia, as may be seen from Figs. 15 and 16, lie close together, with but a few layers of small prothallial cells between them, and with their necks lying freely exposed, during these early stages, at the more or less flat or convex surface of the top of the prothallium. As the archegonia continue their development, the upper sterile portion of the prothallium grows forward, leaving the archegonia behind. This growth progresses until a considerable cavity or archegonial chamber is formed immediately over each archegonium, as shown in Figs. 24 and 25. A similar depression of the archegonia occurs in *Torreya californica* (Robertson, '04), also in *Taxus* (Jäger, '99).

At a very early stage in the development of the archegonium (Fig. 14), the sterile cells immediately surrounding it become very densely granular and very soon acquire the characteristics of the sheath or jacket-cells so commonly met with throughout the Conifers. These cells increase

in number, as development progresses, and eventually each mature archegonium is completely clothed by a single layer of them. In her description of the jacket-cells for *Torreya californica*, Miss Robertson ('04) merely states that they are more rich in protoplasm than their neighbours and have conspicuous nuclei. For *Torreya taxifolia* Coulter and Land ('05) report that the jacket-cells do not make their appearance until after fertilization, and even then they are not well organized. In *Cephalotaxus* the jacket is a constant and striking feature of the archegonia from the very early stages until they become disorganized by the developing embryo. In view of the fact that Arnoldi ('00) has described and elaborately figured the passage of jacket-cell nuclei through the wall and into the egg-cytoplasm, the behaviour and character of these cells demanded considerable attention. After a careful and detailed study I was unable to detect any evidence of nuclear transference or any indications of perforations in the cell-walls through which the nuclei might pass. The nuclei of the jacket-cells, to all appearances, were perfectly normal and active until the disorganization of the archegonium by the developing embryo. The so-called 'proteid vacuoles' were observed in the egg-cytoplasm some considerable time before fertilization, and during the period of the actual fusion of the male and female nuclei they were particularly abundant. As to their origin I am unable to speak, but it seems highly improbable that they have any direct relationship to the nuclei of the jacket-cells. There is not a common jacket for the group of archegonia as occurs in *Cryptomeria* (Arnoldi, '01; Lawson, '04) and other Cupressineae, but each archegonium is surrounded by its own single layer of nourishing cells, as shown in Figs. 16 and 24. In this regard they more nearly resemble the conditions occurring in the Abietineae.

Previous to the division of the central nucleus the cytoplasm of the archegonium is finely granular and contains numerous vacuoles of various sizes. These latter become so numerous as to give a frothy appearance to the whole mass of cytoplasm. Mr. Boodle tells me he finds identically the same condition in *C. Fortunei.* A very similar appearance has also been figured for the young archegonium of *Picea* by Miyake ('03). The nucleus during this period takes up its position directly under the neck-cells as indicated in Fig. 17. The chromatin is in the spireme condition and there is generally present one and frequently two nucleoli. The nucleus remains in this position during the period of its mitosis, which gives rise to the ventral canal nucleus and egg-nucleus. In this connexion it is worthy of note that Coulter and Land ('05) were unable to detect a ventral canal-cell or nucleus in *Torreya taxifolia*, and express it as their opinion that a division of the central nucleus in this species does not occur. Miss Robertson ('04), on the other hand, reports that in *Torreya californica* she was able to detect two archegonia with

the central nucleus in process of division. In *Cephalotaxus drupacea* there can be no doubt of the division of the central nucleus and the organization of the ventral canal nucleus. I was not only able to find numerous cases showing the ventral canal nucleus (Fig. 23) but also a series of stages clearly showing the formation of the spindle.

The first indication of the division of the central nucleus is the usual enlargement and the condensation of the chromatin thread for the organization of definite chromosomes. During this period of changes within the nucleus, the cytoplasm surrounding it becomes quite dense, and kinoplasmic fibrils soon make their appearance. Fig. 18 represents a stage when the nuclear membrane is partly broken down and the fibrils of the young spindle are quite visible, although the general contour of the nucleus is still retained. Fig. 19 shows a little older stage more highly magnified. Here the elongated chromosomes are clearly defined and one of the poles of the spindle is already indicated by the drawing out and converging of the fibrils towards a definite point. I was unable to detect any differentiated areas of cytoplasm which would even suggest the presence of centrospheres, and feel confident that the spindle is formed in the manner which prevails among the Angiosperms.

Fig. 20 shows this spindle with the chromosomes at the poles, and Fig. 21 shows the same stage more highly magnified. Being the last mitosis in the history of the female gametophyte it will be interesting to compare the chromatin with that of the first mitosis of the sporophyte which immediately follows fertilization, and which is represented in Fig. 35. This, however, will be touched upon again when we describe the details of fertilization. Suffice it to say that the chromosomes are very long bodies and that these are, as nearly as could be estimated, ten in number.

As shown in Fig. 21, the continuous fibrils of the spindle persist between the two masses of chromatin for some little time. It is these fibrils which, in some Conifers, take part in the organization of the cell-plate which separates the ventral canal-cell from the egg. In *Cephalotaxus* these fibrils completely disappear without forming a membrane. A ventral canal membrane is developed in *Pinus* (Blackman, '98; Coulter and Chamberlain, '01; Ferguson, '01), *Tsuga* (Murrill, '00), *Picea* and *Abies* (Miyake, '03). As in many other Conifers, the ventral canal-cell in *Cephalotaxus* is represented only by the nucleus. As indicated in Fig. 22, the egg-nucleus and the ventral nucleus are practically identical as to size and shape. For a short time they occupy a position, one above the other, in the neck region of the archegonium. There seemed to be no special differentiation of the cytoplasm about either of them. There was not a trace of a membrane formed at any time. In fact, I feel tolerably certain none is formed. In this respect *Cephalotaxus* resembles *Juniperus* (Strasburger, '79), *Thuja* (Land, '02), *Taxodium* (Coker, '03), *Cryptomeria* (Lawson,

'04), *Sequoia* (Lawson, '04), *Podocarpus* (Coker, '03), and probably *Torreya* (Robertson, '04). It is an interesting fact that in all those forms so far investigated in regard to the ventral canal-cell, the membrane which separates the egg from the ventral nucleus has only been found in representatives of the Abietineae. This fact itself may not be of great importance, especially as there are many forms yet to be investigated. It seems to be significant, however, when we take it in connexion with the survival of the vestigial prothallial cells in the pollen-grain, for here too these vestigial structures are only found, so far as we know at present, in representatives of the Abietineae (the single exception being that of *Podocarpus*). If the retention of such evanescent vestigial structures as the prothallial cells in the pollen or the membrane of the ventral canal-cell has any phylogenetic bearing, then we have an argument in support of the primitive character of the Abietineae as a group.

Great care was taken to follow up the further history of the ventral nucleus. It remains in the neck region until the egg-nucleus moves down and takes a position in the centre of the archegonium and it then shows signs of disorganization. It completely disintegrates, and deeply-staining nuclear fragments of it were frequently observed just below the neck-cells. At the time of fertilization very little trace of it was left. By the time the ventral nucleus has become disorganized the egg-nucleus is found in the widest part of the archegonium (Fig. 27), and here, much enlarged, it remains until its fusion with the male nucleus.

For a short time before and during fertilization the cytoplasm takes on an extraordinary modification. Peculiar dense centres of cytoplasm make their appearance and, as shown in Figs. 26, 27, and 28, they are arranged in a single row which extends from the egg-nucleus to the base of the archegonium. In a general way they have the appearance of asters, for from each of them the cytoplasm is arranged in a series of radiations. These radiations do not seem to be more fibrous than the rest of the cytoplasm but are peculiar in that they extend out from these definite centres. I am unable to understand the meaning of these structures, but they are a constant and striking feature of the mature archegonia.

Fertilization.

From our description above we have seen how the pollen-tube pushes its way through the nucellar tissue until it reaches the cavity in the female prothallium immediately above one of the archegonia. At this period of its development the tube contains the stalk- and tube-nuclei, lying freely in the cytoplasm, and the two sperm-nuclei, both enveloped in the old wall of the body-cell. All of these structures are at the tip of the tube, which has become greatly distended. In some cases it was found that the contents of

the tube had been discharged into one of the archegonial chambers before the neck-cells had been reached. Whether this early discharge of the tube contents is normal it is difficult to say. It may be due to the bursting of the tube by the fixing fluids, as the tip at this time has all the appearance of being under considerable osmotic pressure. In many cases examined the tip of the tube was carried between the neck-cells and into the archegonium before its contents were liberated. Fig. 25 shows the swollen end of the pollen-tube within the archegonium, and its contents are apparently still intact. As the tip of the tube approaches the neck-cells, the two sperm-nuclei may be separated from each other for a very short distance, but quite as frequently they cling close together until the interior of the archegonium has been reached. In Fig. 27 the tip of the pollen-tube is represented just outside the neck-cell; there is a space between the two sperm-nuclei, and these latter appear to be spherical in form. Arnoldi's ('00) description of fertilization in *Cephalotaxus Fortunei* is very meagre, and from his figures it is difficult to make out just what happens during these stages. In several cases I was able to observe the two male nuclei, after entering the archegonium, to be still enveloped by the membrane of the body-cell, and frequently accompanied by the tube- and stalk-nuclei. From the disturbed condition of the cytoplasm above the egg-nucleus, the discharge of the tube contents and the migration of the sperm-nucleus towards that of the egg must take place with considerable force. This is clearly illustrated in Figs. 28, 29, 30, and 31.

It seems to be a common occurrence among the Coniferales for the entire contents of the pollen-tube to enter a single archegonium. According to Blackman ('98), Coulter and Chamberlain ('01), and Ferguson ('01), the pollen-tube itself does not enter the archegonium in *Pinus*, but its entire contents are forced between the neck-cells, and are later found in the cytoplasm of the egg. Practically the same thing occurs in *Taxodium*, *Picea*, and *Abies* (Coker, '03; Miyake, '03), and a similar condition has recently been reported for *Torreya taxifolia* by Coulter and Land ('05). Exceptions to this are found in *Torreya californica* (Robertson, '04) and in *Sequoia* (Lawson, '04), where but a single sperm-nucleus enters the egg. From Belajeff's ('91) figures it seems that this may also be true for *Taxus*. In *Cryptomeria* (Lawson, '04) a single male cell enters the archegonium.

Among the Gymnosperms there seems to be a decided tendency to modify and reduce the structure of the male gametes. In the Cycads and *Ginkgo* we have the two free-swimming ciliated sperms. In the Cupressineae and Taxodineae there are two distinct male cells of equal size organized, and each is surrounded by an independent cell-wall. In *Taxus*, *Podocarpus*, and *Torreya taxifolia* there are two distinct male cells organized, but one of them is dwarfed and functionless and much smaller than the other. In the Abietineae, *Cephalotaxus* and *Torreya californica*, the two male gametes are

represented only by nuclei, since there is no cell-wall separating the one from the other. It is obvious that this reduction and modification of the male gametes of the Coniferales is working along two distinct lines. One of these is to reduce and practically eliminate one of the male cells, and the other is to reduce the structure of the gametes to nuclei. Inasmuch as the nucleus is the essential part of the gamete, the degeneration of one of the male cells must be regarded as a recent and highly specialized condition.

Returning to the conditions found in *Cephalotaxus drupacea*, it seems that very little time elapses between the entrance of the tube-contents and the dissolving of the membrane which surrounds the two sperm-nuclei within the archegonium. As soon as they are released, one of them immediately advances towards the egg-nucleus. When separated from each other they become perfectly spherical, and there is no perceptible difference in their size, shape, or in the structure of the chromatin ; indeed, it is quite impossible to determine which one is destined to unite with the egg-nucleus until one of them approaches the latter. In one case I observed the two sperm-nuclei in touch with the female nucleus, but whether there was ever an actual triple fusion I was unable to determine. In a large number of preparations I was able to see but one of the sperm-nuclei functioning; the second one invariably remained behind, near the neck-cells. Arnoldi ('00) reports that the functionless sperm-nucleus in *Cephalotaxus Fortunei* may give rise to several smaller nuclei by dividing amitotically. I was unable to confirm this for *C. drupacea*. In one case, during a pro-embryo stage, I found the second sperm-nucleus in the upper part of the archegonium, apparently having undergone no division whatever.

As shown in Fig. 29, the path taken by the advancing sperm-nucleus may be followed by the vacuole-like track in its rear. From the fact that the cytoplasm has not closed in around it as it advances towards the female nucleus, its movement is evidently a rapid one. It enlarges slightly as it approaches the egg-nucleus, and at the time of contact it is about one-third the size of the latter. Fig. 30 shows the sperm-nucleus in contact with the egg-nucleus. As shown in this figure and those following, the conditions are similar to those which occur in *Pinus* and other Abietineae. The sperm-nucleus does not break through the membrane of the egg-nucleus, but forces its way into the latter without losing any of its almost spherical form. Fig. 31 shows the male nucleus almost inside of the female, while the male cytoplasm partially envelops them both. The second male nucleus may be seen in the neck region of the egg-cytoplasm. Fig. 32 shows a little later condition ; here the functioning sperm-nucleus has enlarged slightly, and the chromatin in the egg-nucleus has undergone a change. Fig. 33 shows the fusing nuclei more highly magnified. It will be seen from these figures that there is a very obvious difference in the structure of the

chromatin of the sex-nuclei. The male chromatin is very finely and uniformly granular, while that of the female consists of much larger granules arranged on a network of linin.

In the difference in size of the sex-nuclei at the time of fusion, *Cephalotaxus* resembles the conditions described by Blackman ('98) and others for *Pinus* and the Abietineae in general, but differs from those found in *Sequoia* and *Cryptomeria* (Lawson, '04), where the male nucleus enlarges almost to the size of the female before actual fusion takes place.

The early stages in the formation of the first segmentation-spindle were not found. It seemed perfectly clear, however, that very little time elapses between the fusion of the chromatin masses and the organization of the first spindle. According to Blackman ('98), Chamberlain ('99), and Ferguson ('01), no resting fertilized nucleus is formed in *Pinus*. If such a nucleus is formed in *Cephalotaxus*, its resting period is a very short one. I was unable to identify the male and female chromatin after the stage shown in Fig. 33, but the first segmentation-spindle was frequently met with.

According to Coker ('03), the fusion of the male and female nuclei takes place at the base of the archegonium in *Taxodium*. Jäger ('99) reports a similar condition for *Taxus*. As shown in Fig. 34, this cannot be true in *Cephalotaxus*, for the first cleavage-spindle is always formed in the identical place where the fusion of the nuclei occurred—that is, in the middle region of the archegonium. The first cleavage-spindle, more highly magnified, is shown in Fig. 35. Here the chromosomes are at the equator, and are very long bodies, extending almost to the poles of the spindle. A repeated counting of the chromosomes convinces me that these are twenty in number in the sporophyte, which is twice the number found in the gametophyte.

The Embryo.

Upon the fusion of the sperm-nucleus with that of the egg, the embryonal sporophyte begins its existence. The first cleavage-spindle is immediately organized and its formation takes place within the area occupied by the fusion-nucleus, and, as stated above, this fusion occurs in the middle region of the archegonium. The position of the first spindle is shown in Fig. 34. Its axis is not parallel to the long axis of the archegonium and may even lie at right angles to the latter. The dense granular cytoplasm brought in by the sperm-nuclei may be distinguished until after the first division, and forms a complete zone about the spindle.

The result of the first division of the fusion-nucleus is shown in Fig. 36. There are two free nuclei formed. When first organized they are some distance apart, but very soon approach each other and become enveloped in a common dense sheath of starch and other granular substances. As they lie very close together they might easily be mistaken for the sex-

nuclei in process of fusion, had these latter stages not been carefully studied beforehand (Fig. 36). As soon as they are formed these two free nuclei move towards the base of the archegonium. They travel but a very short distance in this direction, however, before the second division occurs. Each of them divides and we thus have four free nuclei in the pro-embryo. Fig. 37 represents a longitudinal section of the archegonium, at this time showing three of the four nuclei and their relative position to each other. The four of them are grouped very near to each other and are apparently not very far removed from the original position of the fusion-nucleus.

In *Taxus*, Jäger ('99) reports that the free nuclei are organized at the base of the archegonium, and a similar condition apparently occurs in *Podocarpus* (Coker, '02). In *Torreya* the free nuclei are also found at the base of the archegonium, but the formation of walls between the nuclei takes place after the second division (Robertson, '04), (Coulter and Land, '05). It will thus be seen that even in the early stages of the pro-embryo *Cephalotaxus* is unlike either of these types of the Taxaceae, and, as we shall point out, in the later stages the dissimilarity is much more striking.

Very soon after the second division, all of the starch, 'proteid vacuoles,' and other cytoplasmic granules sink to the lower or basal region of the egg, thus dividing the archegonium into two distinct and sharply differentiated parts. The less dense or upper part of the archegonium apparently becomes disorganized and functions no further, while the lower part or basal region becomes occupied with the free nuclei of the pro-embryo. Fig. 38 shows the differentiation of the pro-embryonal region at this time and that the free nuclei do not sink to the extreme base of the archegonium as they do at this stage in so many other Conifers.

A third division of the free nuclei now follows, resulting in the formation of eight of them, and still there is no indication of cell-walls separating them. Fig. 39 shows a pro-embryo at this time; six of the free nuclei are shown as well as their relative position to each other. It will also be observed that the free nuclei are arranged in tiers, two for each tier. The next division is followed by the formation of membranes between the nuclei and the organization of the first cells of the embryo. These cells are arranged in tiers, but this arrangement is not as clearly defined as that which prevails in the Abietineae and Cupressineae, where three sharply defined tiers are present. In certain stages three tiers were formed, and in others four could be distinguished. On account of irregular arrangement of the first cells that are formed and the rapid division that follows, it was difficult to say whether the fourth tier originated from a subdivision of the first tier at the tip or from the uppermost tier of nuclei. As Strasburger ('79) has pointed out, there is a group of terminal cells organized which serves as an organ of penetration. After a careful

study I am inclined to believe that these cap-cells originate from a subdivision of the first tier at the tip of the pro-embryo.

My observations on the development of the embryo proper confirm Strasburger's ('79) account of the embryo of *Cephalotaxus Fortunei*. As shown in Fig. 40 there are four distinct regions to be distinguished. The first is the penetrating cap which soon becomes thrown off, the second is the embryo itself, the third suspensors, and the fourth the rosette. Unlike most Conifers, the cells of the second tier, which constitute the embryo proper, divide very rapidly and become very numerous. There may be as many as sixteen or thirty-two cells formed before there is any perceptible elongation of the suspensors. This early merismatic activity of the embryo may explain the necessity for the development of the terminal cells into a penetrating cap. This explanation finds support in the fact that the cap-cells are thrown off very soon after the suspensors are developed. The cap-cells are in two layers. The first consists of five or six cells not much larger than the embryo-cells, while the second consists of a very large terminal cell, very much elongated and tapering to a point. Very soon after the suspensors have reached the length shown in Fig. 40 the cap-cells are discarded. The stages shown in Figs. 42 and 43 are soon after the cap-cells have been thrown off.

The suspensors now elongate very rapidly and carry the embryo down into the endosperm. The elongation of the suspensor. is at first in a straight line, but as growth proceeds they become more or less twisted or curved. This curvature gradually becomes more marked until a distinct winding and twisting form is assumed. As shown in Fig. 43 the suspensors become many times the length of the archegonium, and on account of their winding growth it is quite impossible to trace them back to their point of origin from a single section.

At an early stage during the elongation of the suspensors there appeared to be an occasional budding from the main group of embryo-cells and the formation of small secondary embryos. These however were not frequently found. As a rule there is but one embryo formed from a single archegonium. The small secondary embryos when they appear do not develop very far. In one case one of these smaller embryos was observed growing towards the neck end of the archegonium, quite in the opposite direction taken by the main embryo. Even when two or three main embryos from separate archegonia develop, one of them takes the lead and grows much more rapidly than the others. Such conditions are shown in Figs. 43 and 44.

The next period in the development of the embryo is marked by the appearance of the embryonal tubes or secondary suspensors and the disorganization of the primary suspensors. The tubes or secondary suspensors were first observed soon after the stage shown in Fig. 43. The

layer of embryo-cells next to the suspensors first elongate and this is followed by the second, third, fourth, and so on in regular series. Several layers of the embryo-cells thus become very much elongated, and continue the function of the primary suspensors in carrying the tip of the embryo deeper down into the tissue of the endosperm. Fig. 44 shows an advanced stage in the development of the embryo. It will be observed from this figure, that the elongation of these embryonal cells may exceed the length of the primary suspensors and that the first of them are the longest and appear gradually shorter as they approach the growing tip of the embryo.

Summary and Conclusions.

At the time of pollination the microspore consists of two cells—the tube-cell and the generative cell. Vestigial cells or nuclei representing the vegetative prothallial cells of the male gametophyte are entirely lacking.

Pollination takes place late in March. Three or four pollen-grains become lodged on the top of the nucellus at the base of the micropyle, and while they enlarge considerably they show no further nuclear activity until the following spring.

There are usually three or four pollen-tubes produced. At the time of the penetration of the tube into the nucellar tissue, the generative nucleus divides, giving rise to the body-cell and the stalk-nucleus. The tube thus contains one large cell and two free nuclei.

It takes about ten days for the tip of the tube to reach the archegonial chamber. When this has been reached, the body-cell divides, giving rise to two sperm-nuclei. The two sperm-nuclei are of equal size, and no cell-wall is formed between them. There are therefore not two male *cells* formed, but simply two large nuclei which lie close together within the membrane of the body-cell.

Only one megaspore germinates, which gives rise to the female prothallium. The megaspore first enlarges, and this is followed by free nuclear division. A central vacuole soon makes its appearance, and by increasing in size, forces the cytoplasm and free nuclei to the wall. During the growth of the vacuole, free nuclear division proceeds at a rapid rate. The presence of a megaspore-membrane could not be detected in the young prothallium, but during the later fertilization-stages an extremely thin membrane was observed. The prothallium very soon consists of a large central vacuole and a very thin parietal layer of cytoplasm in which the free nuclei are distributed at intervals.

When the parietal layer has reached a certain thickness a series of walls are formed between the free nuclei, thus constituting the primary cells of the prothallium which are open and exposed to the sap of the vacuole on the inner side. By their inward growth the primary cells eventually close

up the space occupied by the sap of the vacuole. Before the vacuole is completely closed however, numerous cross-walls are formed.

As soon as the prothallial tissue is organized the archegonial initials make their appearance in the form of superficial cells at the apex. There are generally two, but frequently three, cells formed in the neck of the archegonium. The sterile tissue around the necks of the archegonia grows forward leaving the archegonia behind, thus forming four distinct cavities or archegonial chambers. There are four archegonia organized, and each is surrounded by a single layer of jacket-cells. There was no evidence to show any migration of the jacket-cell nuclei into the archegonium.

A distinct ventral canal-nucleus is organized, which in size, shape, and chromatin contents resembles that of the egg. It degenerates before fertilization takes place.

The entire contents of the pollen-tube enter the egg. The two sperm-nuclei are not released from the membrane of the body-cell until the interior of the archegonium has been reached. The sperm-nuclei are perfectly similar, and it is impossible to say which one will function until one of them moves towards the egg-nucleus. The second male nucleus remains behind in the neck region of the archegonium.

The fusion of the sex nuclei takes place in the middle of the archegonium, and at this time the male is about one-third the size of the female. If a resting fertilized nucleus is formed, its resting period is very brief, for the first cleavage spindle is organized immediately after fusion.

The first division of the fusion nucleus takes place in the middle of the archegonium. The first division is immediately followed by a second which results in the formation of four free nuclei in the pro-embryo.

After the second division, all of the starch 'proteid vacuoles' and other cytoplasmic granules sink to the lower part of the egg, and the archegonium thus becomes sharply differentiated into two distinct regions. The lower dense region becomes occupied by the free nuclei of the pro-embryo.

By repeated free nuclear division there are sixteen nuclei organized before the formation of cell-walls. The cells eventually become arranged in four tiers. The end tier develops into a penetrating cap, the second tier forms the embryo proper, the third the suspensors, and the uppermost the rosette.

There may be as many as sixteen or thirty-two cells in the embryo before there is any perceptible elongation of the suspensors. This early merismatic activity of the embryo-cells may account for the necessity of organizing a penetrating cap from the terminal cells. Soon after the suspensors have developed the penetrating cap is thrown off.

When the primary suspensors have reached their full length their function is continued by a series of long embryonal tubes or secondary suspensors which are developed from the proximal cells of the embryo.

Several cases of budding from the main group of embryo-cells were found, resulting in the formation of small secondary embryos, but as a rule one archegonium produces but a single embryo.

From this account of the gametophytes it becomes obvious that *Cephalotaxus* cannot be regarded as a primitive type of Conifer, although this is contrary to results obtained from certain studies on the sporophyte. From his investigations on the vascular structure of the ovule Worsdell ('00) regards *Cephalotaxus* as the most ancient of the Coniferous genera, and concludes that 'this genus forms in some measure a connecting link between Cycadaceae and Coniferae, and helps us to trace, however faintly, a fragment of the line of descent of the latter group.' By comparing the gametophytes of *Cephalotaxus* with the Cycadales and with other Coniferales I cannot accept Worsdell's view. In fact I am forced to the conclusion that this genus represents a very recent type of Conifer.

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LITERATURE CITED.

ARNOLDI, W. ('00): Beiträge zur Morphologie der Gymnospermen: III. Embryogenie von *Cephalotaxus Fortunei*. Flora, lxxxvii, p. 46, 1900.

——— ('00): Beiträge zur Morphologie einiger Gymnospermen: I. Die Entwicklung des Endosperms bei *Sequoia sempervirens*. Bull. Soc. Imp. Nat. Moscou, 1900.

——— ('00): Beiträge zur Morphologie und Entwicklungsgeschichte einiger Gymnospermen: II. Ueber die Corpuscula und Pollenschläuche bei *Sequoia sempervirens*. Bull. Soc. Imp. Nat. Moscou, 1900.

——— ('01): Beiträge zur Morphologie einiger Gymnospermen: V. Weitere Untersuchungen über die Embryologie in der Familie der Sequoiaceen. Bull. Soc. Imp. Nat. Moscou, 1901.

——— ('00): Beiträge zur Morphologie der Gymnospermen: IV. Was sind die 'Keimbläschen' oder 'Hofmeisterskörperchen' in der Eizelle der Abietineen? Flora, lxxxvii, p. 1, 1900.

BELAJEFF, W. ('93): Zur Lehre von dem Pollenschlauche der Gymnospermen. Ber. der Deutsch. Bot. Gesell., xi, p. 196.

BLACKMAN, V. H. ('98): On the Cytological Features of Fertilization and Related Phenomena in *Pinus silvestris*, L. Phil. Trans. Roy. Soc., cxc, p. 395.

CHAMBERLAIN, C. J. ('99): Oogenesis in *Pinus Laricio*. Bot. Gaz., xxvii, p. 268.

COKER, W. C. ('02): Notes on the Gametophytes and Embryo of *Podocarpus*. Bot. Gaz., xxxiii, p. 89.

——— ('03): The Gametophytes and Embryo of *Taxodium*. Bot. Gaz., xxxvi, pp. 1–27 and 114–40.

——— ('04): On the spores of certain Coniferae. Bot. Gaz., xxxviii, p. 206, 1904.

COULTER, J. M., and CHAMBERLAIN, J. C. ('01): Morphology of Spermatophytes, Part I. New York, 1901.

COULTER, J. M., and LAND, W. J. G. ('05) Gametophytes and Embryo of *Torreya taxifolia*. Bot. Gaz., xxxix, p. 161, 1905.

DIXON, H. N. ('94): Fertilization in *Pinus silvestris*. Ann. Bot., viii, p. 21.

FERGUSON, M. C. ('01): The Development of the Pollen-tube and the Division of the Generative Nucleus in certain species of *Pinus*. Ann. Bot., xv, p. 193.
———— ('01): The Development of the Egg and Fertilization in *Pinus Strobus*. Ann. Bot., xv, p. 435.
GOROSCHANKIN, J. ('83): Zur Kenntniss der Corpuscula bei den Gymnospermen. Bot. Zeit., xli, p. 825.
HIRASÉ, S. ('95): Études sur la fécondation et l'embryogénie du *Ginkgo biloba*. Jour. Coll. Sci. Imp. Univ. Tokyo, viii, p. 307.
IKENO, S. ('96): Das Spermatozoid von *Cycas revoluta*. Bot. Mag. Tokyo, x, p. 367.
———— '98): Entwickelung der Geschlechtsorgane und Vorgang der Befruchtung bei *Cycas revoluta*. Pringsheim's Jahrbuch für wiss. Bot., Bd. xxxii, 1898.
JÄGER, L. ('99): Beiträge zur Kenntniss der Endospermbildung und zur Embryologie von *Taxus baccata*. Flora, lxxxvi, p. 241.
JUEL, H. O. ('00): Beiträge zur Kenntniss der Tetradentheilung. Jahrb. f. wiss. Bot., xxxv, p. 626, 1900.
———— ('04): Ueber den Pollenschlauch von *Cupressus*. Flora, xcili, p. 56, 1904.
LAND, W. J. G. ('02): A Morphological Study of *Thuja*. Bot. Gaz., xxxvi, p. 249.
LAWSON, A. A. ('04): The Gametophytes, Archegonia, Fertilization and Embryo of *Sequoia sempervirens*. Ann. Bot., xviii, no. lxix, p. 1, 1904.
———— ('04): The Gametophytes, Fertilization and Embryo of *Cryptomeria Japonica*. Ann. Bot., xviii, no. lxxi, 1904.
LOPRIORE, G. ('05): Ueber die Vielkernigkeit der Pollenkörner von *Araucaria Bidwillii*. Ber. der Deutsch. Bot. Gesell., Bd. xxiii, 1905.
MIYAKE, K. ('03): On the Development of the Sexual Organs and Fertilization of *Picea excelsa*. Ann. Bot., xvii, p. 66.
———— ('03): Contribution to the Fertilization and Embryogeny of *Abies balsama*. Beih. zum Bot. Cent., xiv, p. 134, 1903.
MURRILL, W. A. ('00): The Development of the Archegonium and Fertilization in the Hemlock Spruce (*Tsuga Canadensis*). Ann. Bot., xiv, p. 583.
ROBERTSON. A. ('04): Spore formation in *Torreya californica*. New Phyt., iii, p. 133, 1904.
———— ('04): Studies in the Morphology of *Torreya californica*. New Phyt. iii, p. 205, 1904.
SHAW, W. R. ('96): Contribution to the Life-history of *Sequoia sempervirens*. Bot. Gaz., xxi, p. 332.
SOKOLOWA, MLLE C. ('90): Naissance de l'endosperme dans le sac embryonnaire de quelques Gymnospermes. Bull. Soc. Imp. Nat. Moscou, 1890 (1891), p. 446.
STRASBURGER, E. ('72): Die Coniferen und Gnetaceen, 1872.
———— ('79): Die Angiospermen und die Gymnospermen, 1879.
———— ('84): Neue Untersuchungen über die Befruchtungsvorgänge bei den Phanerogamen als Grundlage für eine Theorie der Zeugung. Jena, 1884.
———— ('92): Ueber das Verhalten des Pollens und die Befruchtungsvorgänge bei den Gymnospermen. Hist. Beit., iv, p. 1, 1892.
THOMSON, R. B. ('05): The megaspore-membrane of the Gymnosperms. Univ. Toronto Studies, Biol. series, iv, 1905.
WEBBER, H. J. ('97): Peculiar Structure in the Pollen-tube of *Zamia*. Bot. Gaz., xxiii, p. 453.
———— ('97): The Development of the Antherozoids of *Zamia*. Bot. Gaz., xxiv, p. 16.
———— ('97): Notes on the Fecundation of *Zamia* and the Pollen-tube Apparatus of *Ginkgo*. Bot. Gaz., xxiv, p. 225.
———— ('01): Spermatogenesis and Fecundation of *Zamia*. U.S. Dept. Agri. Bur. of Plant Industry. Bull., ii, 1901.
WORSDELL, W. C. ('00): The Vascular Structure of the Ovule of *Cephalotaxus*. Ann. Bot., xiv, p. 317, 1900.
WOYCICKI, Z. ('99): Fertilization in the Coniferae. Warsaw, 1898. Reviewed in Jour. Roy. Micro. Soc., p. 482, 1900.

EXPLANATION OF FIGURES IN PLATES I—IV.

Illustrating Dr. Lawson's Paper on *Cephalotaxus*.

All figures were drawn with the aid of the camera Lucida. The following oculars and objectives were used.

Figs. 1, 2, 6, 7, 8, 12, 19, 21, Zeiss oc. 1, obj. oil imm. $\frac{1}{12}$.
Figs. 3, 44, Zeiss oc. 2, obj. 3.
Figs. 4, 5, 9, 10, Zeiss oc. 3, obj. 3.
Figs. 11, 13, 14, 15, 16, 24, Zeiss oc. 1, obj. 3.
Figs, 17, 18, 20, 22, 26, 27, 28, 29, 30, 31, 32, 34, 36, 37, 38, 39, 40, 41, 42, 43, Zeiss oc. 6, obj. 3.
Fig. 25. Zeiss oc. 4, obj. 3.
Fig. 23. Zeiss oc. 4, obj. 7.
Fig. 33. Zeiss oc. 2, obj. oil imm. $\frac{1}{12}$.
Fig. 35. Zeiss oc. 6, obj oil imm. $\frac{1}{12}$.

Fig. 1. A cross-section of a microspore some time before pollination, showing the single large nucleus and the presence of starch granules. March 7, 1904.

Fig. 2. A cross-section of a microspore just before pollination showing the tube and the generative nucleus.

Fig. 3. A longitudinal section of the ovule at the time of pollination showing the megasporangium, integument, and micropyle. April 19, 1904.

Fig. 4. A longitudinal section through the upper part of the nucellus showing a young pollen-tube containing the body-cell B., and the stalk and tube-nuclei *s. t.* May 11, 1904.

Fig. 5. The same ten days later showing a pollen-tube that has completely penetrated the nucellar tissue with the body-cell, and the stalk and tube-nuclei in the distended tip of the tube. May 21, 1904.

Fig. 6. A tip of a pollen tube more highly magnified showing the character of the cytoplasm in the tube and in the body-cell, and nucleus of the latter preparing for division. May 21, 1904.

Fig. 7. The two sperm-nuclei resulting from the division of the body-cell nucleus. They occupy the greater part of the cavity of the body-cell, the old membrane of which still envelops them. The stalk and tube-nuclei are also present. May 23, 1904.

Fig. 8. Two sperm-nuclei showing the characteristic way in which they fold over each other. May 26, 1904.

Fig. 9. A longitudinal section of a very young prothallium. The single embryo-sac consists of a thin parietal layer of cytoplasm and a large central vacuole.

Fig. 10. The same as above at a later stage.

Fig. 11. A later stage in the development of the prothallium showing the primary prothallial cells growing in and closing the vacuole.

Fig. 12. A highly magnified section of a part of the parietal layer of cytoplasm taken from the stage represented in Fig. 9.

Fig. 13. A longitudinal section through the upper region of a young prothallium showing a young archegonium with one neck-cell. April 27, 1904.

Fig. 14. A longitudinal section as above with two neck-cells in the archegonium and a single layer of jacket-cells already organized.

Fig. 15. A cross-section through the neck region of the archegonia. Two of the four show three cells in the neck, the remainder only two. May 21, 1904.

Fig. 16. A cross-section through the middle region of the archegonia showing their characteristic perfectly circular form in section and each enveloped in a single layer of jacket-cells. May 21, 1904.

Fig. 17. A longitudinal section of an archegonium with the central nucleus preparing for division. May 23, 1904.

Fig. 18. A longitudinal section of an archegonium with the central nucleus in the early stage of division. Spindle formation has begun. May 26, 1904.

Fig. 19. A more highly magnified section of the central nucleus during spindle formation. The long chromosomes are sharply defined and one pole of the spindle is already indicated by the conveying of the fibrils toward a definite point. The nuclear wall is partly broken down. May 26, 1904.

Fig. 20. A longitudinal section of an archegonium with the central nucleus in an advanced stage of mitosis, the chromosomes being at the poles of the spindle. May 21, 1904.

Fig. 21. The same spindle more highly magnified showing the character of the chromosomes and the absence of any cell-plate formation between the daughter-nuclei. May 21, 1904.

Fig. 22. A longitudinal section of an archegonium showing the ventral canal nucleus and egg-nucleus fully organized and the absence of a membrane separating them. May 30, 1904.

Fig. 23. A longitudinal section of the upper portion of an archegonium more highly magnified, illustrating the similarity of the ventral canal and the egg-nuclei and their relative position just after their organization. May 30, 1904.

Fig. 24. A longitudinal section of two mature archegonia showing their relative position to the archegonial chamber above, and the character and extent of the jacket-cells. May 23, 1904.

Fig. 25. A longitudinal section showing two pollen-tubes one of which has extended into the archegonium. May 30, 1904.

Fig. 26. A longitudinal section of a mature archegonium just before fertilization showing the peculiar cytoplasmic centres in the region below the nucleus. May 26, 1904.

Fig. 27. A longitudinal section showing the two sperm-nuclei still surrounded by the membrane of the body-cell and about to enter the archegonium. The egg-nucleus has enlarged considerably. May 26, 1904.

Fig. 28. A longitudinal section showing one sperm-nucleus within the archegonium, the second sperm-nucleus was observed in the section immediately following. They were both within the wall of the body-cell. May 28, 1904.

Fig. 29. A longitudinal section showing one of the sperm-nuclei approaching the egg-nucleus. Its path and rapid approach is indicated by the disturbed condition of the cytoplasm in its rear May 28, 1904.

Fig. 30. A longitudinal section showing the actual union of one of the sperm-nuclei with the egg-nucleus while the second sperm-nucleus remains in the cytoplasm above. May 26, 1904.

Fig. 31. A longitudinal section showing a similar condition with the enveloping of the fusing nuclei by the male cytoplasm. The sperm-nucleus enters the egg-nucleus without losing its spherical form. May 26, 1904.

Fig. 32. A longitudinal section showing a little later stage in the fusing of the sex-nuclei; the male chromatin being in a less compact condition. May 28, 1904.

Fig. 33. A section of the fusing-nuclei more highly magnified showing the difference in the structure of the male and female chromatin. May 26, 1904.

Fig. 34. A longitudinal action showing the spindle of the division of the fusion-nucleus. The male cytoplasm forms a complete zone around the spindle, but some distance from it. May 23, 1904.

Fig. 35. A more highly magnified section of the first spindle of the sporophyte showing the increased number of chromosomes as compared with those found in the gametophyte. May 23, 1904.

Fig. 36. A longitudinal section showing the first two nuclei of the pro-embryo. They both lie embedded in a dense area of starch granules. May 23, 1904.

Fig. 37. A little later than above showing a four-nuclei stage of the pro-embryo. May 23, 1904.

Fig. 38. The same somewhat later showing all the granular substances of the cytoplasm accumulated in the lower part of the archegonium. May 23, 1904.

Fig. 39. A pro-embryo of eight free nuclei and still no trace of cell wall forming between them.

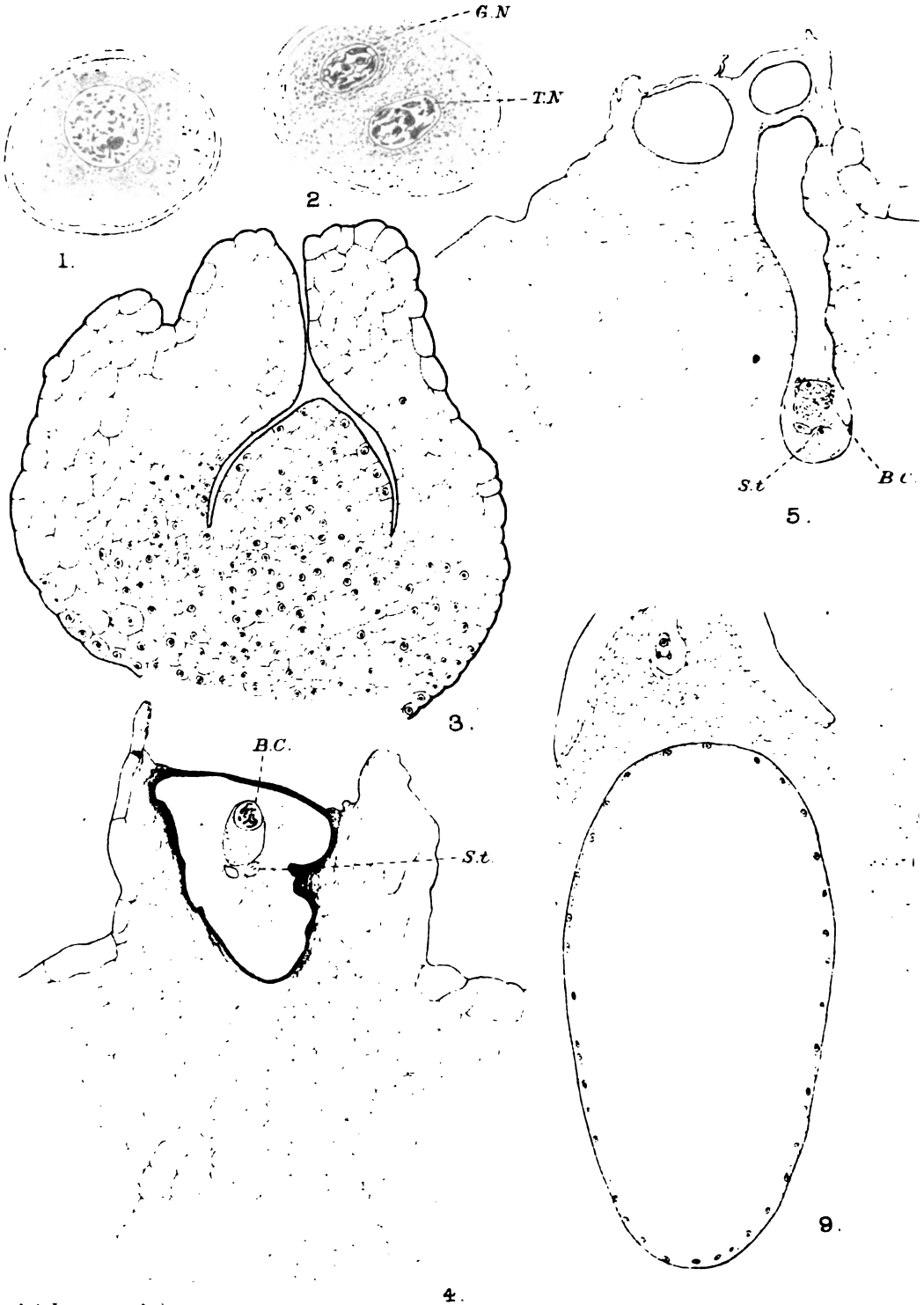
Fig. 40. An older embryo showing the young suspensors and the embryo proper at the ends with the terminal penetrating cap.

Fig. 41. The same as above but slightly older.

Fig. 42. The same still older. The cells forming the penetrating cap have been thrown off.

Fig. 43. An embryo showing the very much elongated and twining suspensors with three younger embryos above.

Fig. 44. A much older embryo. The primary suspensors can no longer be seen, but the secondary suspensors developed from the rear cells of the embryo proper are conspicuous. These secondary suspensors elongate enormously and carry the embryo at the end deep into the endosperm.



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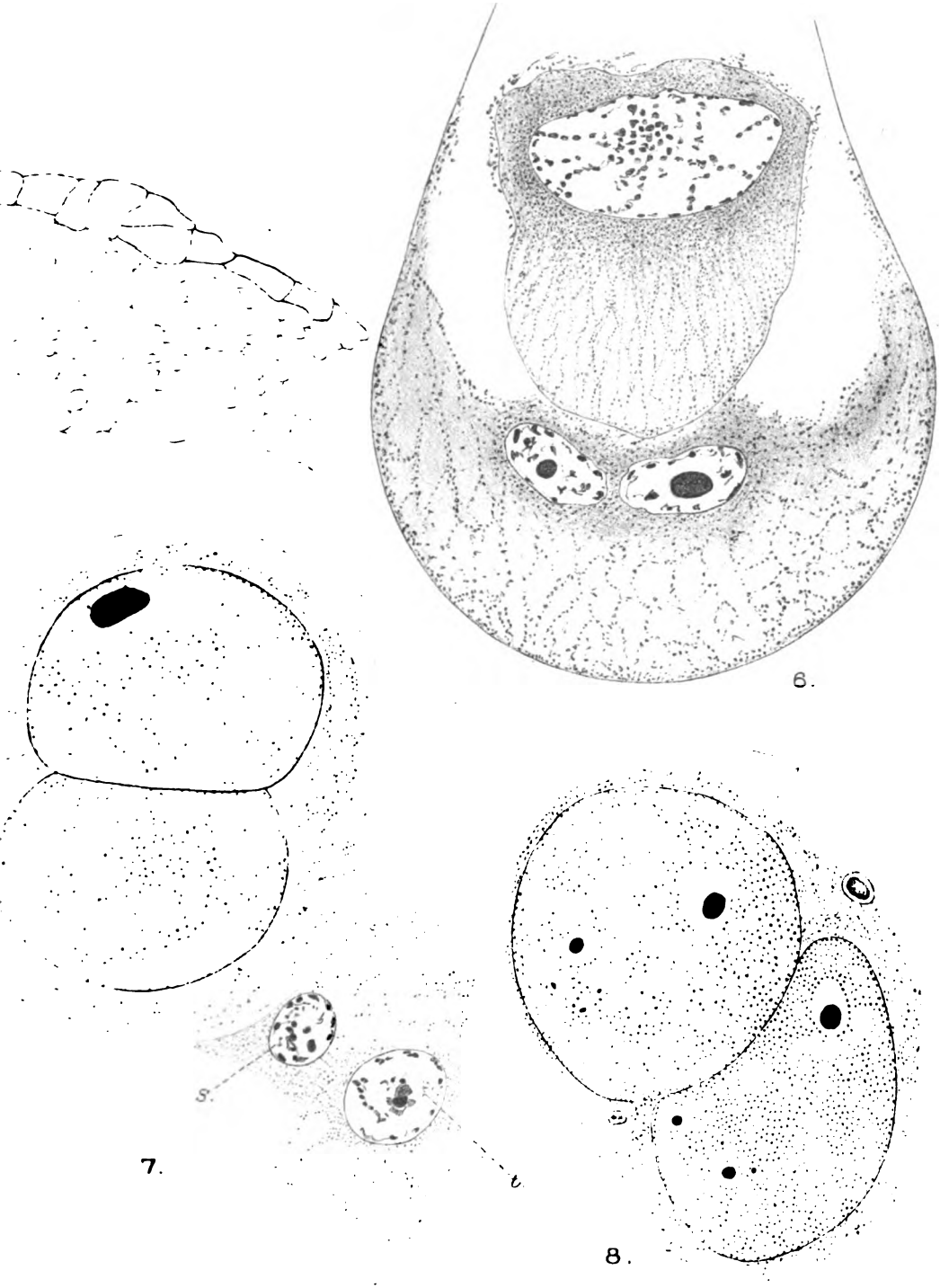

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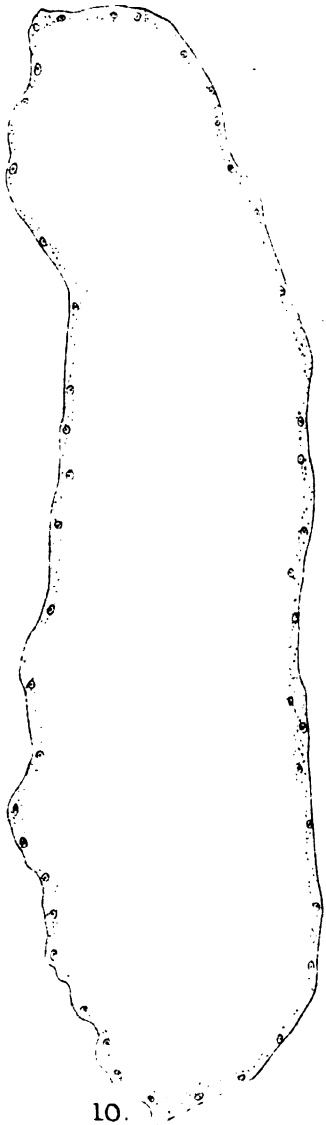

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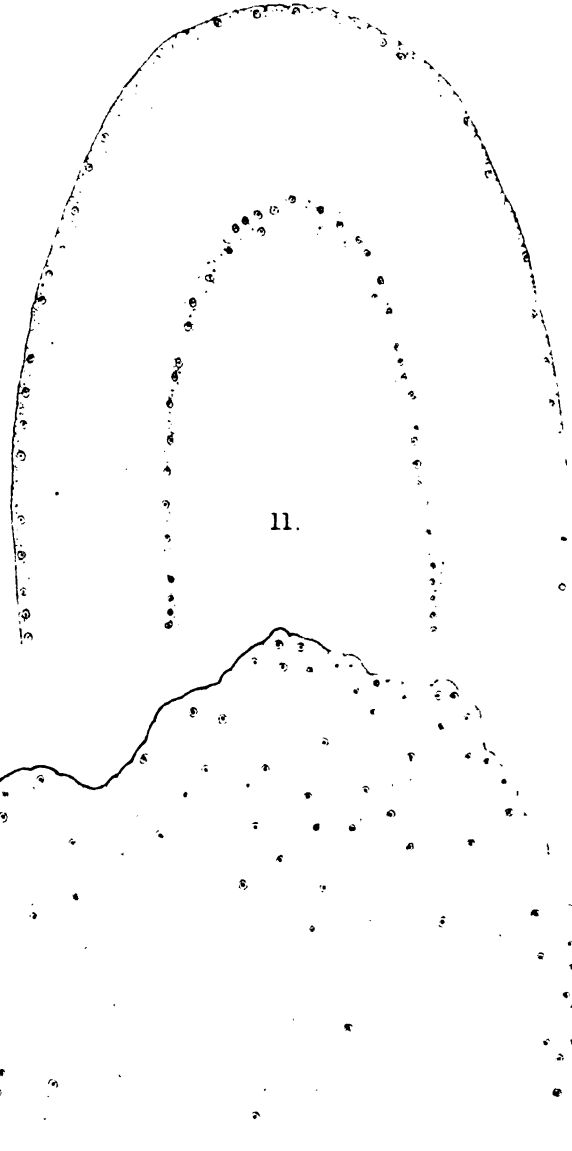

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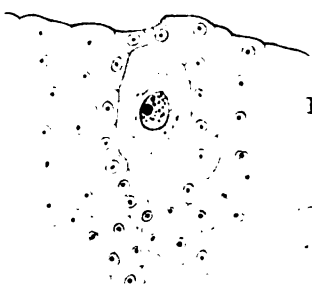

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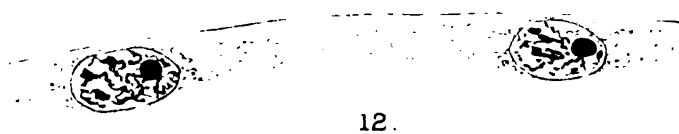

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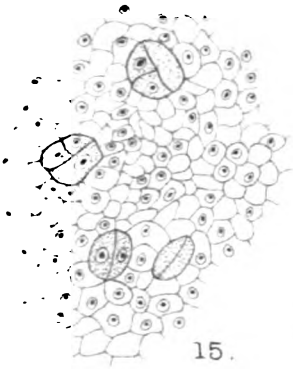

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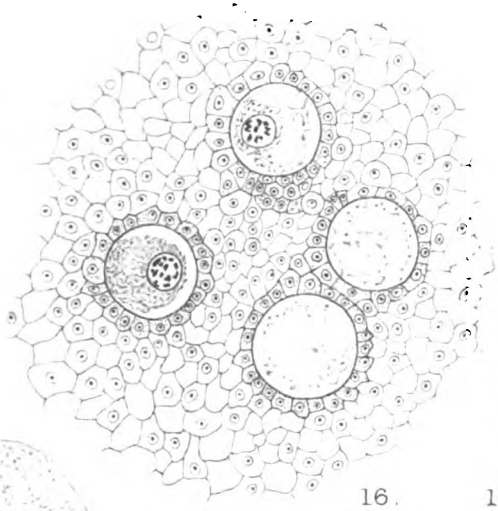

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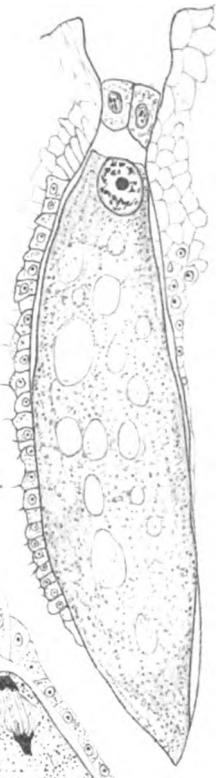

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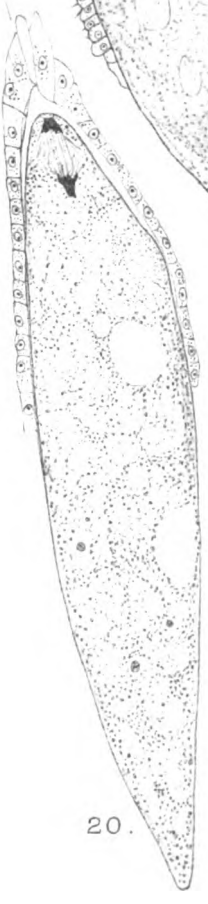

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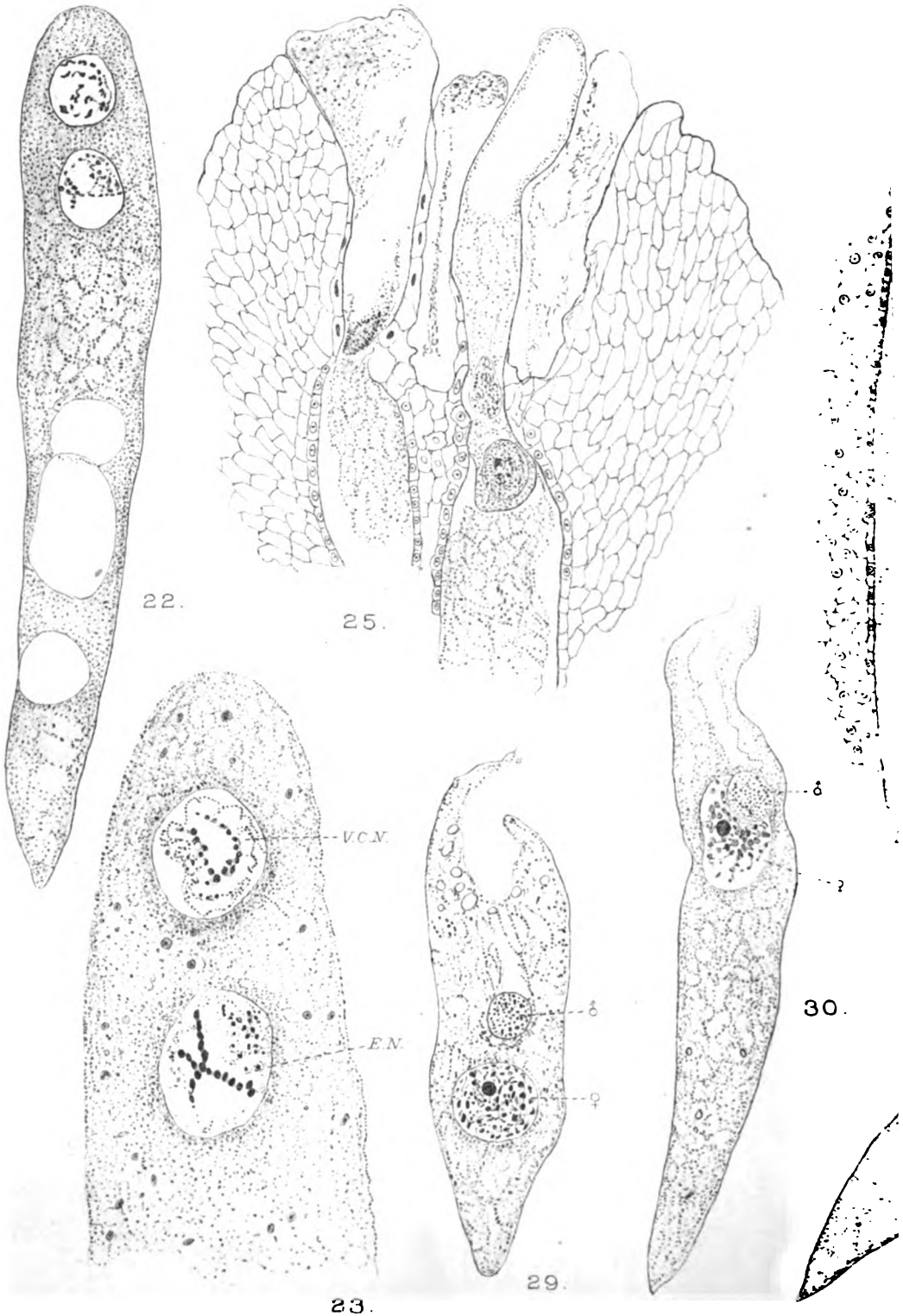

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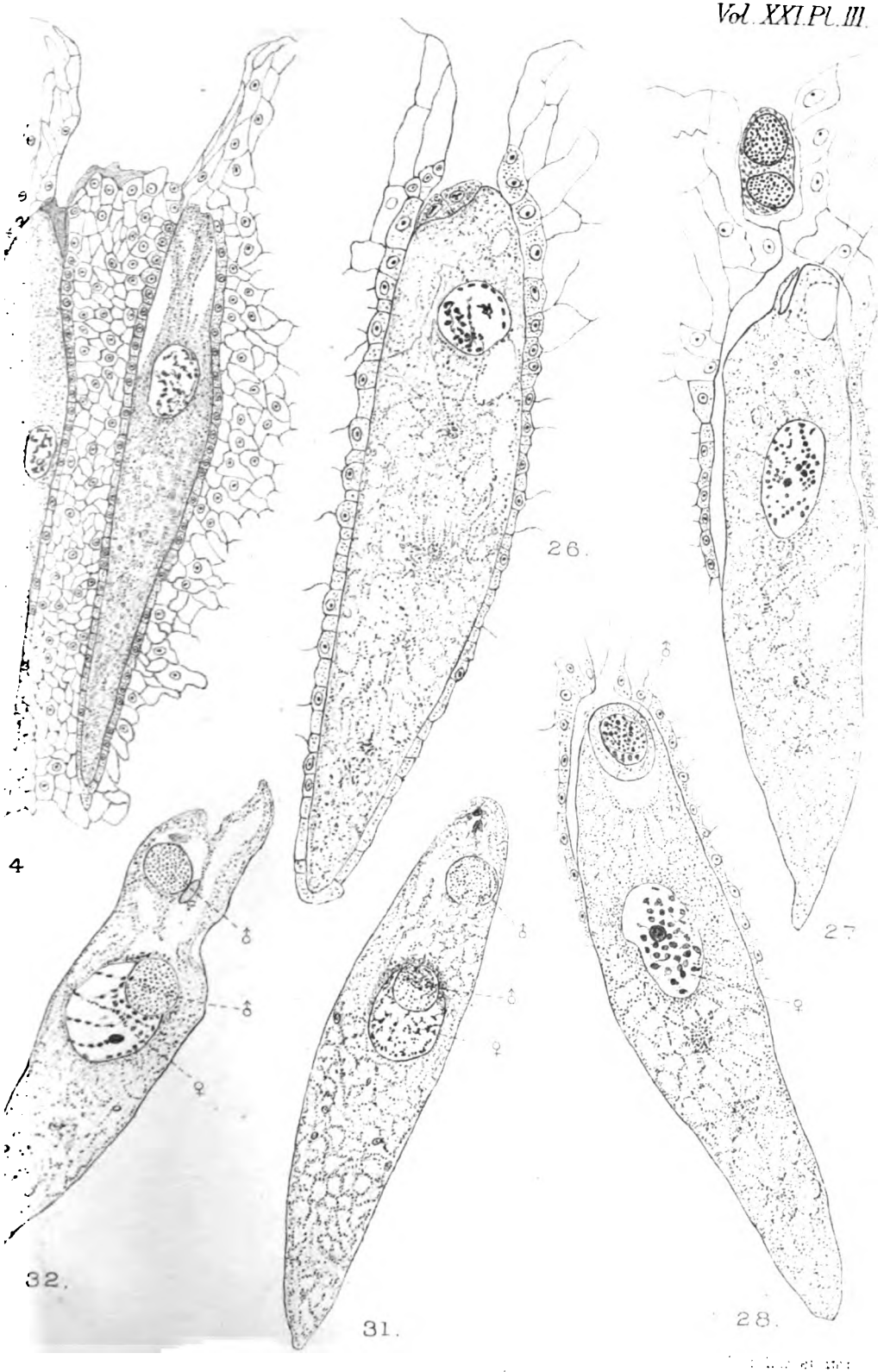
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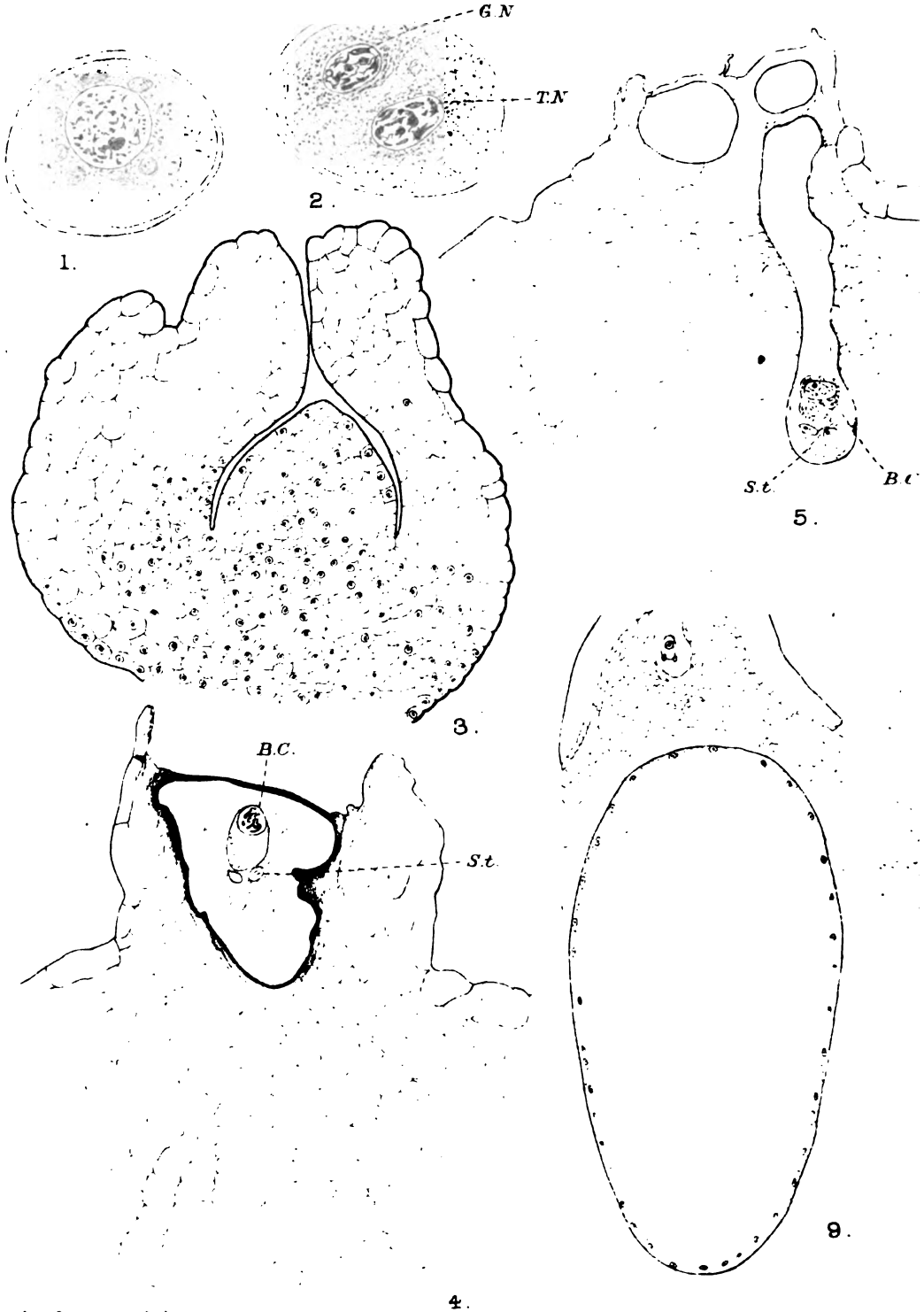

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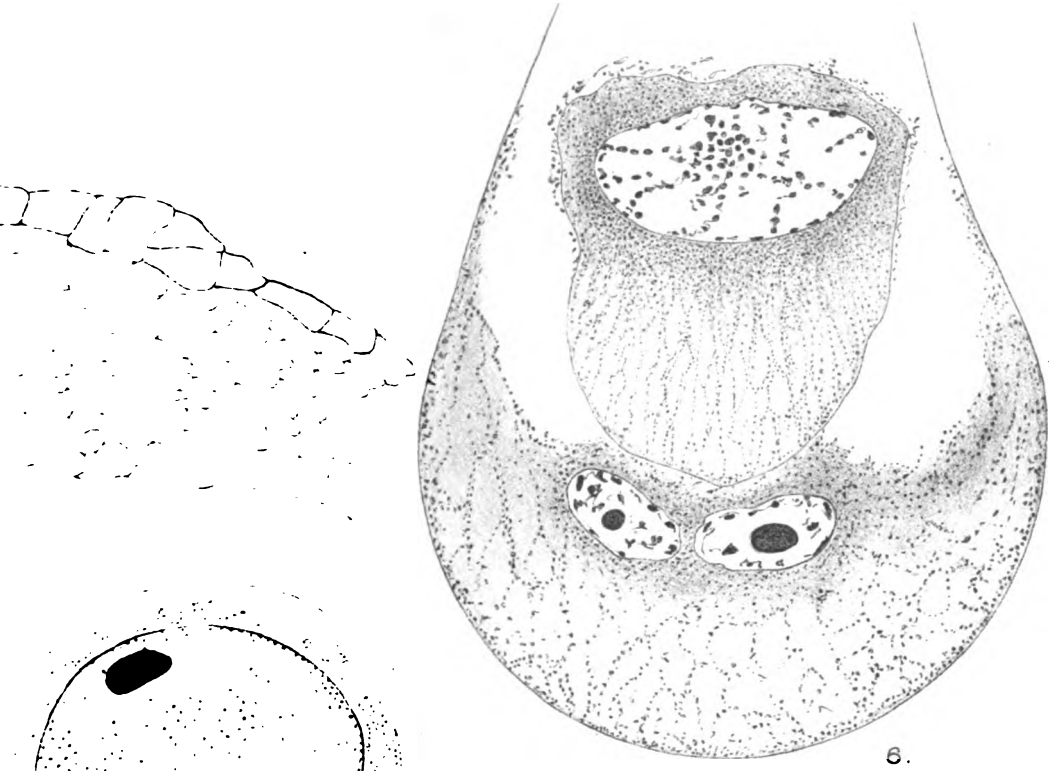

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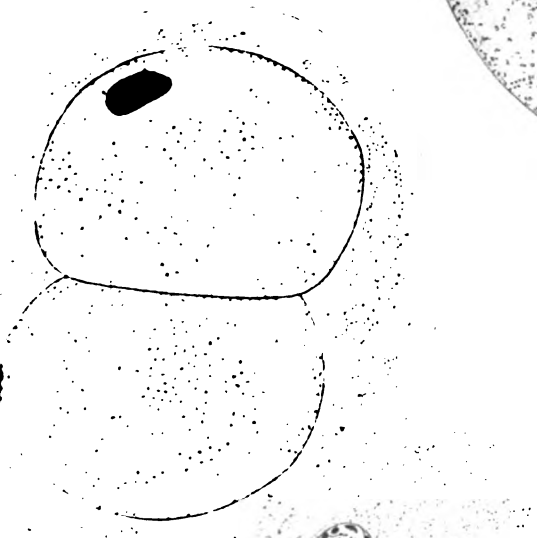

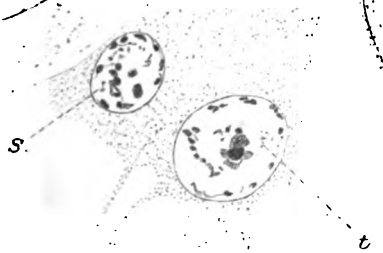

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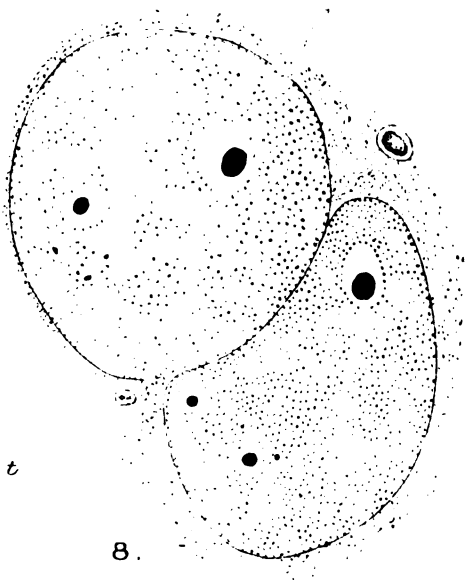

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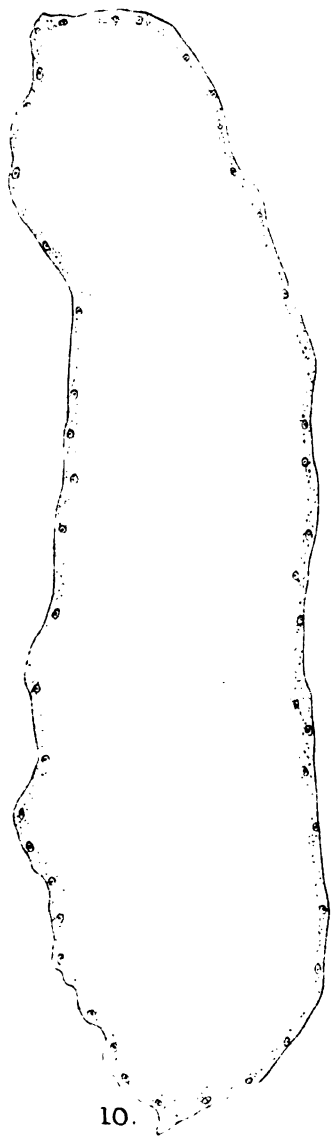

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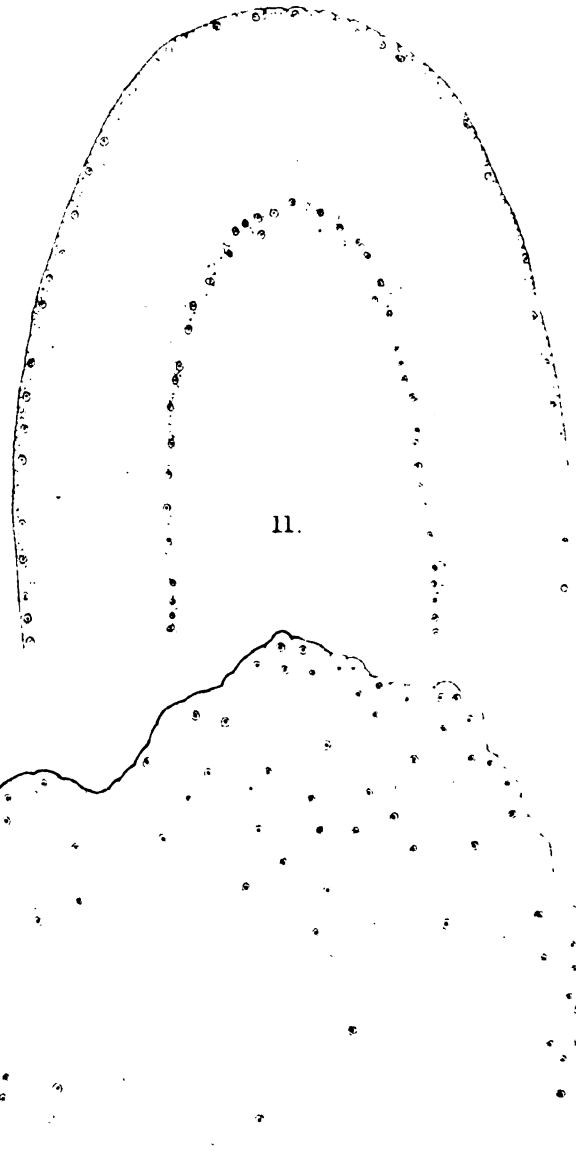

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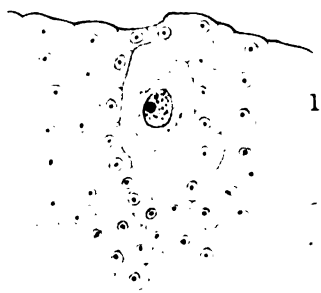

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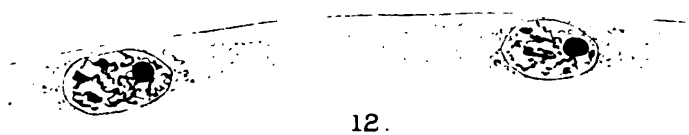

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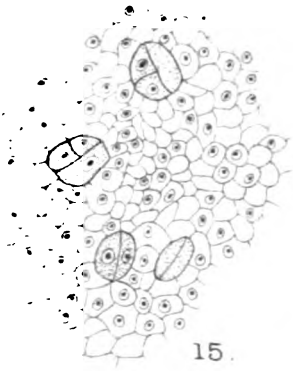

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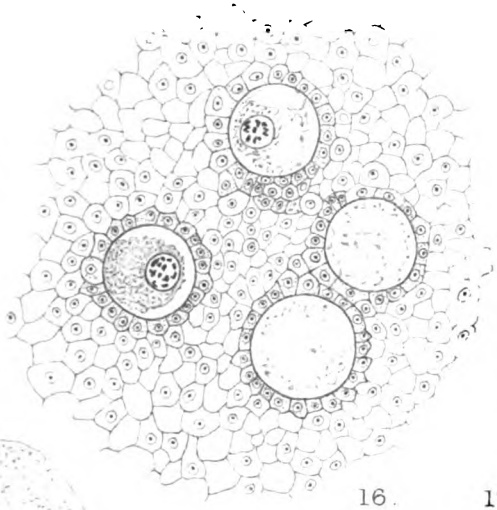

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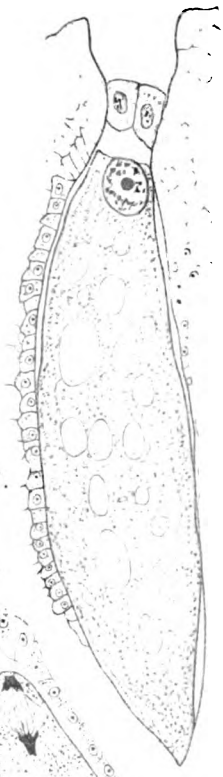

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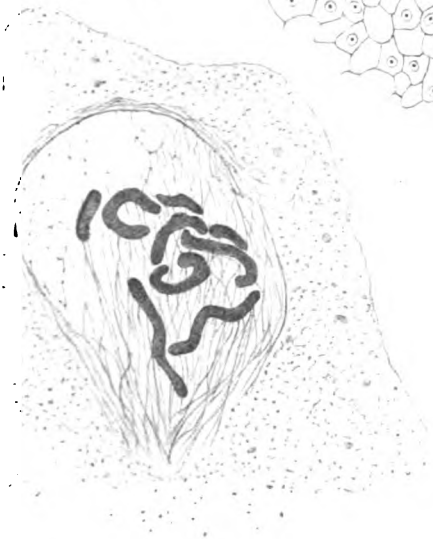

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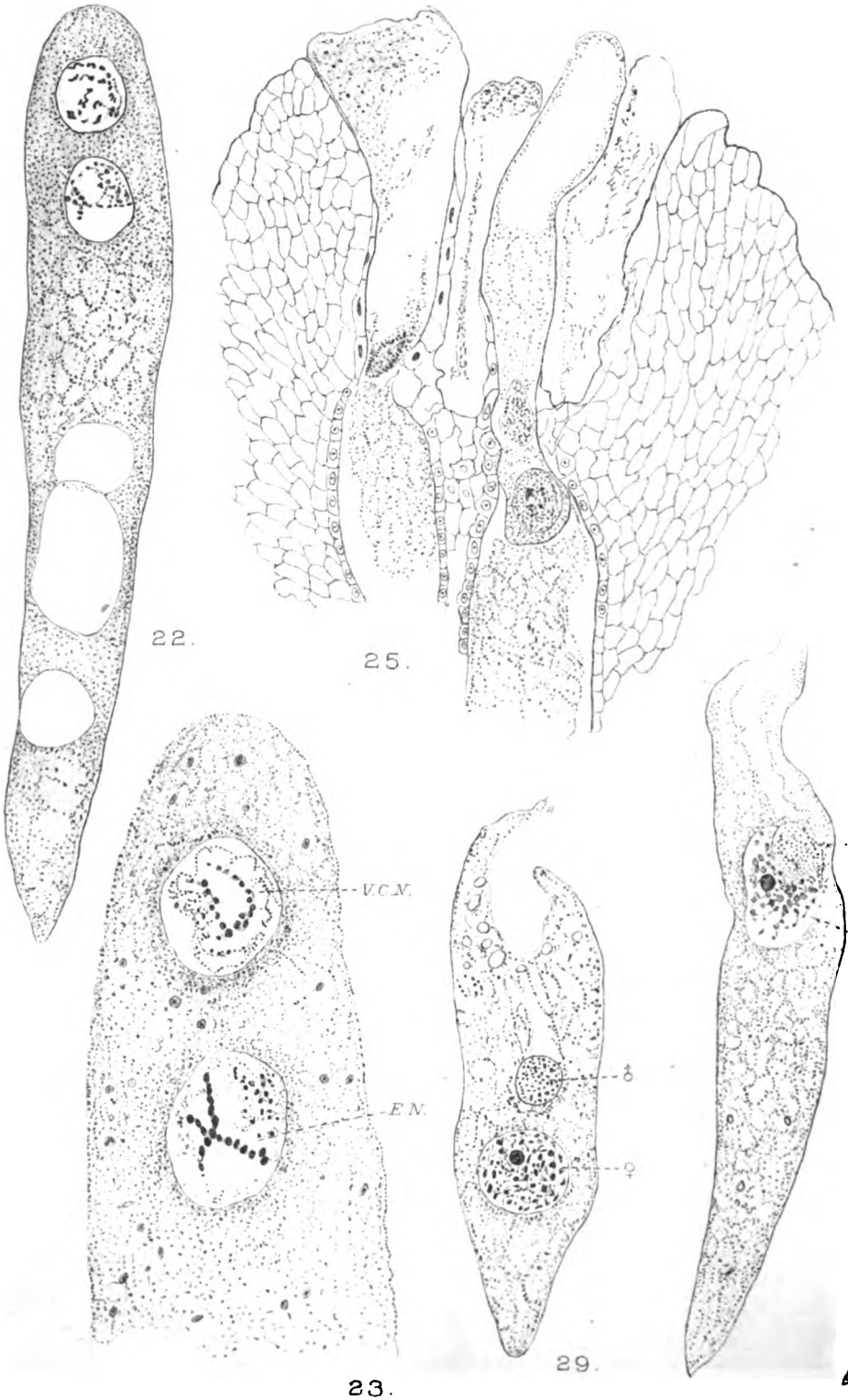

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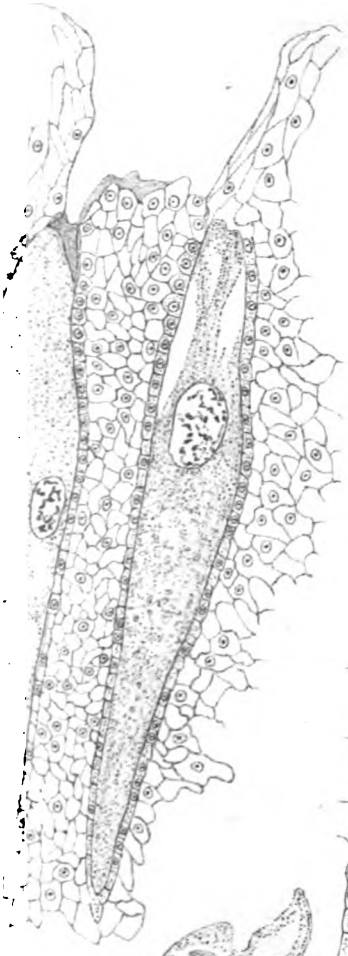

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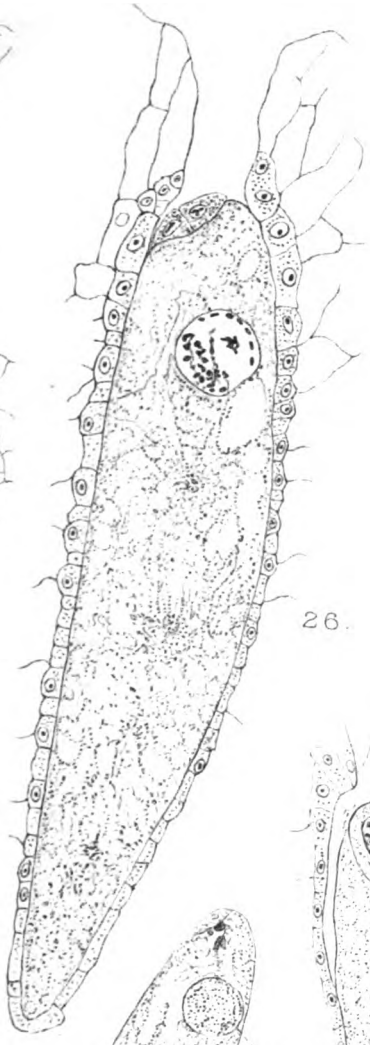

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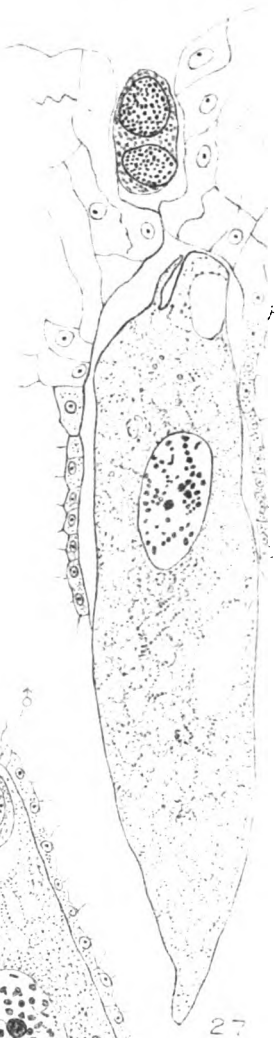

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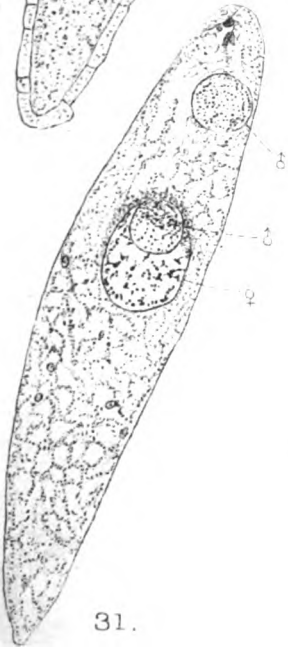

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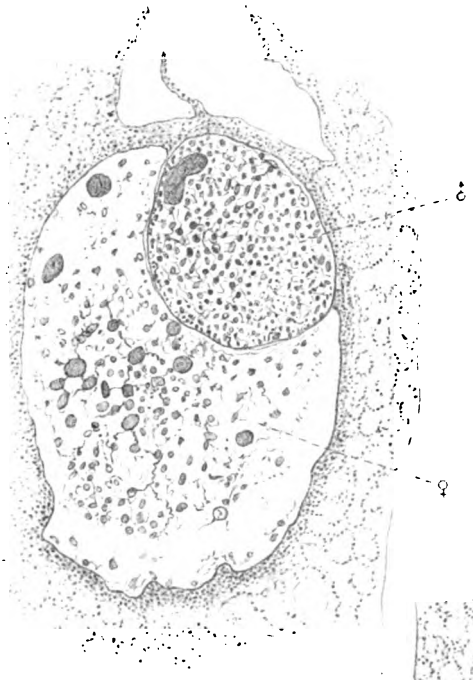

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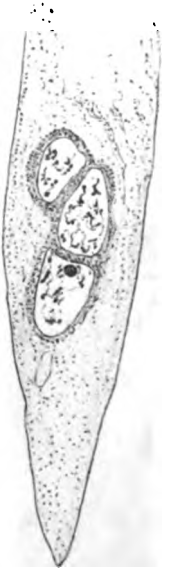

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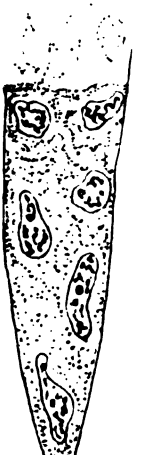

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LAWSON – CEPHALOTAXUS DRUPACEA.

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Notes on the Development and Structure of the Seed in the Alsinoideae.

BY

L. S. GIBBS, F.L.S.

With Plates V and VI and four Figures in the Text.

INTRODUCTION.

THE Caryophyllaceae, from the point of view of embryological development, seemed to have been rather overlooked in recent years, in comparison with the large amount of work which has been done in this particular line of research for other families and individual species.

This was not the case with the older botanists. Schleiden and Vogel (2), Meyen (3), Tulasne (5), and Hofmeister (6) all record interesting conclusions on the morphological development of the suspensor, embryo and embryo-sac. But most of this work, good as it is, is incidental or comparative, and there is no consecutive account of the embryology and development in any one species.

In this order the abundance and persistence of the nucellar perisperm is a marked characteristic, and the formation of this tissue has been followed with interest by several authors. Schleiden and Vogel (2) correctly figure the peculiar shape of the embryo-sac and the localization of the starch storage tissue, describing the former as growing in horseshoe shape round the main mass of the nucellus (perisperm) of which it destroys only the peripheral layers. Hegelmaier (10) defines the limits of the permanent nucellar or perisperm tissue in this order, as the incidental result of working on the morphology of the endosperm in both the groups Silenoideae and Alsinoideae which constitute it. He does not suggest any possible physiological relation between these tissues, and describes endosperm formation in these ovules as transitory in character.

Recently Johnson (23, 25, 26) has worked out very thoroughly the embryology and germination of certain Piperaceae, and one of the chief results of his investigations is to bring out the important rôle played by the endosperm in the development of the embryo.

He draws some interesting conclusions from this fact on the function of the endosperm in all seeds containing abundant perisperm. In *Peperomia pellucida* and in *Heckeria* the endosperm is described as bursting out of the

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seed-coat and continuing to jacket the embryo, which at germination is only an undifferentiated mass of cells, until the root, hypocotyl, and cotyledons are organized. This endosperm is not a storage tissue, but digests the perisperm reserves and passes them on to the embryo. The suggestion is therefore made that this restriction of the function of the endosperm obtains in all seeds with abundant perisperm, the sporophyte of the second generation being nourished, not by the parent generation, but by the intervening gametophyte.

A chance series of sections through a mature seed of *Stellaria aquatica* which seemed to prove the justice of this point of view, led to the present investigation which rather confirms Johnson's hypothesis.

To understand the organization of the mature seed, it was necessary to trace the separate tissues composing it to their origin, and the subsequent results seemed sufficiently interesting to justify publication.

Great uniformity and simplicity of structure prevails in all members of the Alsinoideae examined.

This fact renders the progressive and comparative development of the nucellar tissues, in conjunction with that of the embryo-sac and embryo, easy to follow. Some stress has been laid on this point, as the part played by the separate tissues of the ovule in the development of the embryo are especially accentuated in this case, owing to the early laying down and abundance of the perisperm coupled with the restriction of the functions of the suspensor and endosperm.

Morphologically these ovules are characterized by the constant presence of two integuments, each composed of two layers of cells, the inner integument always projecting beyond the outer one. The nucellus increases in size by the periclinal and anticlinal divisions of the epidermal layer, and this results in the sinking of the embryo-sac in the nucellar tissue, and the formation of a sort of transitory beak at the apex of the nucellus by the outgrowth, prior to fertilization, of certain cells which are subsequently reabsorbed.

The chief feature in the embryology is the filamentous suspensor, the basal cell of which (that directed towards the micropyle) attains to a very large size.

The uniformity that characterizes the endosperm in these ovules is very striking, and one of the chief objects of this research is to determine the function of the cells of this tissue in relation both to their morphological differentiation, and to the nutrition of the embryo.

Variation being so slight in the tribe Alsinoideae, one species is taken as a type.

Stellaria media, L., was chosen as offering a good example, and it was studied as far as the maturation of the seed, partly because the basal suspensor cell reaches its maximum development in this species.

In the stages of early development investigation is difficult owing to the peculiar orientation of the ovules and the position of the loculi. Transverse sections are useless and good longitudinal ones a question of luck. For drawings of these stages, therefore, where better preparations were available in other species they have been substituted for *Stellaria media*. As the ovules mature, gradual changes take place in the seed coat, which result in cuticularization of the cell-walls and infiltration of tannin into both the cell-walls and contents of the tegumentary layers. The tannin is very resistant to the penetration of paraffin and good microtome series are difficult to obtain. For the study of the germination of the seed, *Cerastium perfoliatum* was found to offer a favourable example, as it germinates easily, and the walls of the seed coat are not so cuticularized and contain less tannin than in most other species.

Three distinct stages seem to mark the comparative development of these ovules, viz.:—

1. Pre-fertilization.
2. Post-fertilization to maturation.
3. Germination of the seed.

The descriptive matter has been arranged accordingly.

In this course of development two long rests occur:—

(*a*) in the pre-fertilization stage, immediately after the fusion of the two polar nuclei into one definitive nucleus;

(*b*) on the maturation of the seed.

In explanation of the terms employed, *primary megaspore* stands for the megaspore mother-cell, which develops directly into the embryo-sac, since no subsequent tangential divisions of the primary megaspore cell were observed. The development of the embryo-sac is thus similar to that obtained in many lilies. *Primary suspensor* refers to the whole of the filamentous row of cells preceding the actual embryological divisions, which in the early stages is usually called the pro-embryo.

As the inner and outer integuments are each composed of two layers of cells, these layers are referred to as layer 1 and 2 of the inner integument and layer 1 and 2 of the outer integument respectively, starting from the periphery of the ovule. The functions of these two layers, being dissimilar in the case of the outer integument and similar in that of the inner, it is obviously necessary to differentiate between them.

The lower portion comprises the base of the nucellus and the chalaza.

The species investigated were as follows:—

A. As far as the definitive nucleus stage.

1. Alsineae.

Stellaria Holostea, L., *S. media*, Cyr., *S. graminea*, L., *S. uliginosa*, Murr., *Cerastium glomeratum*, Thuill., *C. quaternellum*, Fenzl,

C. perfoliatum, L., *Sagina apetala*, L., *S. procumbens*, L., *Moehringia*, sp.

2. Sperguleae.

Spergula arvensis, L., and *id.* var. *sativa* (Boenn), *Spergularia rubra*, Pers.

B. From the definitive nucleus stage as far as the maturation of the seed.

1. Alsineae.

Stellaria Holostea, L., *S. media*, Cyr., *S. aquatica*, Scop., *Cerastium glomeratum*, Thuill., *C. perfoliatum*, L., *Sagina apetala*, L., *Alsine trinervia*, L.

2. Sperguleae.

Spergula arvensis, L.

C. Germination of the seed.

1. Alsineae.

Stellaria Holostea, L., *S. aquatica*, Scop., *Cerastium perfoliatum*, L., *Alsine laricifolia*, Wahlenb., *A. fasciculata*, M. Koch.

2. Sperguleae.

Spergula arvensis, L., and *id.* var. *sativa* (Boenn), *Spergularia salina*, Presl.

Historical.

The results of former work on the group are as follows :—

Grew (1) in 1682 figures the seeds of *Spergula* and chickweed, describing the former as 'spherick in shape with a knobbed surface and membranous Rimm,' and the latter as kidney-shaped.

Schleiden and Vogel (2) in 1839, in a paper on 'Albumen' first distinguished between 'perispermium' or storage tissue derived from the nucellus and 'endospermium' or tissue derived from the embryo-sac. They give a very good figure of *Spergula pentandra* with the small celled suspensor which characterizes the Sperguleae, and in a series show starch storage tissue limited to the central mass of the nucellus, which alone persists as the embryo matures.

Meyen (3), 1841, working on *Stellaria media*, noticed the elongation of the suspensor beyond the embryo-sac peculiar to this species. He figures the characteristic twist of the pollen-tube, where it adheres to the apex of the embryo-sac. Following Schleiden, he interprets it as the beginning of the 'vésicule embryonnaire' which he describes as developing first into the apical portion of the suspensor and ultimately into the suspensor and embryo. He considered, that from its size, the suspensor must absorb food material for the embryo.

Unger (4), 1855, figures the style of *Stellaria media*, with a pollen-

grain on a papilla of the stigma, through the wall of which it is supposed to penetrate.

Tulasne (5), 1855, in a most beautiful series of drawings from dissections of the embryo-sac, with embryos in different stages, of *Cerastium triviale* and *C. collinum*, *Holosteum umbellatum* and *Stellaria media*, dwells on the peculiar form of suspensor (vésicule embryonnaire) in the latter, calling the prolongation the 'appendice.' He figures the twist of the pollen-tube at the apex of the embryo-sac, and the persistence of the same long after fertilization as general for species investigated, which differ chiefly in relative size and shape of suspensor.

He describes the suspensor in *Spergula arvensis* and *Spergularia rubra* as much simpler and almost uniform in diameter.

Hofmeister (6), 1858, in *Stellaria media* saw the two synergideae and oosphere (Keimbläschen), but no antipodals, and spoke of the upper ends of the 'Keimbläschen' as being pressed against the 'spitze Ausstülpung, welche die flache Scheitelwölbung des Embryosackes in ihrer Mitte trägt.' He noticed the persistence of the synergideae (unfertilized 'Keimbläschen') till the first division of the fertilized one, when they are quite 'verdrängt' so that only the upper portion of the first cell of the pro-embryo (Keimbläschen) occupies the 'Ausstülpung' of the embryo-sac.

He described the suspensor (Embryoträger) as long in all Caryophyllaceae and the endosperm as scanty and as appearing late.

Vesque (9), 1878, working on the development of the embryo-sac in Angiosperms, found that in *Stellaria Holostea*, the primary mother-cell was hypodermal in origin, developing directly into the embryo-sac without further tangential divisions. He also figures the development of a 'nucellar cap' by increased periclinal divisions.

Guignard (18), 1882, in *Silene obtusifolia* saw two tangential peripheral divisions in the mother-cell, but admits not being able to trace real succession, owing to the slight differentiation between them and the rapid enlargement of inferior cell into the embryo-sac.

Godfrin (15), 1880, working on the seed coats of Angiosperms found such marked uniformity of structure in the Caryophyllaceae (Sileneae and Alsineae) as to be characteristic of the tribe. He figures the seed coat of *Spergula arvensis* in transverse section.

Hegelmaier (11), 1885, in his paper on the Morphology of the Endosperm of Dicotyledons, places the Caryophyllaceae in his third class of 'einseitig peripherischen,' in which the endosperm first lays down one layer at the micropylar end, then develops centripetally, filling up the apical portion of the embryo-sac.

Working on *Stellaria Holostea* for the Alsinoideae, he denies free cell formation at the chalazal end, where the endosperm nuclei merely degenerate, and describes the apical tissue as lasting only for a short period.

The same author in another paper (12), 1895, on the 'Orientation of Embryos in Dicotyledons,' shows that the curvature of the embryo in the Curvembryae is due, in the first instance to no mechanical pressure, as enclosed for a long period in transitory endosperm, it touches neither perisperm nor testa. He mentions the small celled suspensor of *Spergula arvensis*, the spiral position of the cotyledons in the mature seed for that species and their thick and narrow consistency.

Holfert (20), 1890, on the proteid layer (Nährschicht) in *Stellaria nemorum*, mentions three layers as composing the testa of the seed, viz. (1) an epidermis, with wavy cuticle and contracted protoplasm in places; (2) a 'Pigmentschicht' of tangentially stretched cells with brown contents; and (3) a layer of cells bulging towards the inside, parenchymatous and without contents, consequently 'Nährschicht,' as the contents must have been absorbed.

But in *Spergula arvensis* he gives the sequence of the three layers, as (1) epidermis (growing out at intervals into club-shaped hairs), (2) 'Nährschicht,' brown and obliterated, and (3) colourless quadrate cells (in transverse sections) with pitted walls and brown contents.

He worked on mature seeds only.

Balfour (27), 1901, in his comprehensive address on the Angiosperms, throws out some illuminating suggestions as to the function of the integuments as an integral portion of the sporangium, apart from their ultimate purely protective use in the ripe seed.

He describes the tegumentary system of the ovule as an outgrowth of the sporangial primordium of variable origin and development, its primary function in Angiosperms is regarded as being that of water jacket and food store, developed in response to special demands for water involved in the seed habit.

Finally, Johnson (26), 1902, in the germination of the seeds of certain Piperaceae describes the formation of the endosperm, and calls attention to the fact that it is not a storage region, but digests and passes on food material to the embryo from the more abundant perisperm or storage tissue, and he suggests that this same relation between perisperm and endosperm obtains in all seeds with abundant perisperm, such as the Polygonaceae, Chenopodiaceae, Phytolaccaceae, and Caryophyllaceae.

Comparative Development of the Nucellus and Embryo-sac, as far as Fertilization.

Stellaria media.

To study the growth of the nucellus, the very earliest stages in the development of the flower must be examined. After the laying down

of the carpels on the flower rudiment, a conical portion forming the extreme apex of the latter remains, and it is this axile apical portion which grows on in the centre of the carpels, forming the columella. The growth of the columella is at first more rapid than that of the carpellary whorl. The ovules arise on it in basipetal succession, and the ovular outgrowths appear on the top of the columella before it is enclosed by the carpels. The nucellus first consists of a one-layered epidermis and some hypodermal cells. As it increases in length by anticlinal divisions of these cells a larger hypodermal cell is soon distinguishable (Pl. V, Fig. 2, *m.*) terminating the axile row of the nucellus. This cell is the primary megaspore, and as Vesque found for *Stellaria Holostea*, it becomes the functional megaspore without further tangential divisions. Anticlinal divisions now appear in some of the epidermal cells of the nucellus, which if occurring over the megaspore, may simulate tangential divisions of the latter (Pl. V, Figs. 3 and 5). In *Stellaria uliginosa* in two cases, exceptions to this rule were seen (Figs. 1 and 2, *t.* and *m.*) ; but in Fig. 2 the apparent tapetum may be derived from the epidermal layer, the section being possibly oblique.

As the primary megaspore enlarges, two or three of the cells below it in the same vertical row become differentiated from the surrounding tissue in size, denser contents, and in larger nuclei (Pl. V, Figs. 3 and 5, *ax. c.*), and it is at the expense of these cells that the subsequent growth in length of the megaspore takes place.

The cells of the nucellus in immediate contact with the megaspore also show larger nuclei and denser contents, simulating sporogenous tissue. Some caution is therefore necessary in the interpretation of even slightly oblique sections.

The integuments arise in basipetal succession.

Embryo-sac. The first division of the nucleus of the megaspore occurs before the inner integument encloses the nucellus (Pl. V, Fig. 5, *e. s.*). Subsequent divisions to the eight-nuclei stage follow in normal sequence (Fig. 6, *e. s.*). Very rapid anticlinal, and less rapid periclinal, divisions of the epidermal layer of the nucellus follow (Pl. V, Fig. 6, *per. l.*), with the result that the embryo-sac becomes sunk in its tissue and is enclosed in four or five concentric layers which join on to the axile rows at the base of the nucellus (Figs. 6 and 7, *per. l.* and *ax. c.*).

Increased anticlinal divisions occur at the apex of the nucellus (Fig. 4, *ap. nuc.*), also noted by Vesque (9), who speaks of the 'nucellar cap.' The increased periclinal divisions he figures for the 'cap' were not seen, and each layer in every case can be traced all round the periphery of the nucellus in all stages of the growing ovule. These cells, at the immediate apex of the nucellus just under the micropyle, form loose vertical rows (Figs. 6, 7, *ap. nuc.*), the extreme cells of which, just before fertilization, are prolonged as papillae into the micropyle (Fig. 13, *ap. nuc.*).

The embryo-sac after elongating at the expense of the primary axile row of cells (Figs. 5, 6, 7, *e. s.*), expands by the absorption of the limiting concentric nucellar layer, which in this stage shows starch contents (Figs. 6 and 7, *per. l. st.*). Progressive digestion of these limiting layers is shown by the existence, in contact with the embryo-sac, of disorganized cells, which remain in the position of, and can be traced back to, the layer of which they formed part (Pl. V, Figs. 6 and 7, *dis. c.*).

At this stage the embryo-sac contains eight free nuclei, and these quickly differentiate into the two synergidae and oosphere, three antipodals and two polar nuclei (Fig. 6, *syn. oos. ant. p. n.*). The cells of the nucellus in the immediate vicinity of the antipodals present the same unattached and partially digested appearance as those surrounding the rest of the embryo-sac (Figs. 7 and 8, *ant.*). No evidence points to the antipodals influencing the solution of tissue in contact with them. They are ephemeral, disappearing after fertilization; and even before their differentiation the disintegrating changes in the nucellus are apparent. This seems to show that the actual cytoplasm of the embryo-sac is the active digestive agent up to fertilization, and also to a certain extent afterwards.

The synergidae are well defined. They are long cells, and contain large nuclei (Fig. 7, *syn.*).

Fusion of polar nuclei. The fusion of the two polar nuclei into one definitive endosperm-nucleus occurs some time before fertilization (Fig. 7, *d. n.*). The definitive nucleus resulting from the fusion is very large, with a well-marked nucleolus and dense reticulum. It occupies the centre of the embryo-sac towards the upper portion, and is in contact with the oosphere. The latter, which lies against the synergidae and near the embryo-sac wall, is smaller with a more alveolate reticulum (Fig. 7, *oos.*).

The fact that the nuclear membranes of the definitive nucleus and oosphere are in contact is characteristic of *Stellaria media* (Fig. 9, *d. n.*). In other species examined, this was never found to be the case, some cytoplasm always intervening (Pl. V, Fig. 8, *d. n.*). At this stage a very long rest occurs. It is on that account the most easy to obtain, and was found to occur in sections both of the expanding bud and open flower.

Progressive development of endosperm, perisperm, and embryo after fertilization. The pollen-grains settle on the papillae of the stigma, as was correctly figured by Unger (4), the tubes growing *along* the cell-walls, but not *penetrating* them as he describes. They continue to force their way between the cell layers composing the tissue of the style which is in direct continuity with that of the septa of the ovary.

These septa consist of loose, spongy tissue which forms papillae on each surface, and it is on these papillae that the micropyles of the ovules abut. This fact explains the definite orientation of the two rows of ovules in each loculus.

Miss Lister (19) notes the spongy nature of the septa in this group, and suggests their probable function as conducting-tissue for the pollen-tubes. In the course of the present investigation, tubes were repeatedly seen in the septa of the ovary, and also penetrating through the papillae which clothe their surface, to the micropyles of the ovules. These papillae persist after fertilization, and, as the septa break down as the ovules increase in size, the ruptured surface on the columella becomes covered by similar outgrowths of the cells. They evidently serve to ensure the nutrition, and form the conducting-tissue for the pollen-tubes in their passage to the ovules. In *Arenaria tenuifolia* these papillae elongate considerably, entirely filling up the cavity of the ovary. They replace to a certain degree the septa, all of which become broken down by the growth of the ovules, and possibly they may serve as paraphyses to keep the ovules damp.

As the pollen-tube makes its way through the conducting-tissues of the style and septum of the ovary to the ovule, some modifications take place in the latter. The contents of the fan-like rows of cells forming the extreme apex of the nucellus, as previously described on p. 31, show increased density and darker staining properties, and the terminal cells of these rows grow out as long papillae into the micropyle (Fig. 13, *ap. nuc.*).

The inner integument projects far beyond the outer, and the cells of which this projecting portion is composed show considerable increase in size and darker staining properties (Fig. 7, *i. i.*). The contents of these cells are used up by the growing pollen-tube, the walls shrink, leaving a cavity, and it is this cavity that the papillose outgrowths of the nucellar apical layers project.

The function of these cells is probably to ensure the nutrition, and to facilitate the passage, of the pollen-tube to the embryo-sac. Their subsequent absorption by the pollen-tube leaves a channel from the apex of the nucellus to the embryo-sac, in which the tube persists for a long time (Pl. V, Figs. 17 and 18, *p. t.*).

Before entering the synergidae the pollen-tube forms a slight swelling, and the apex then forces its way between them and lies against the oosphere (Fig. 9, *p. t.*). Further penetration into the cavity of the embryo-sac was not observed. The pollen-tube in all other species of the Alsinoideae examined is very thick and persistent, forming a very characteristic twist on itself before penetrating the synergidae (Figs. 13, 17, *p. t.*). But *Stellaria media* forms an exception to this rule. Tulasne (5), in his drawings of dissections of the embryo-sac with suspensor and embryo, figures the twist adhering to the apex of the embryo-sac in every other species examined by him, and he lays stress on the fact that it was impossible to dissect out the one without the other. In *Stellaria media*, however, this was not the case, and he found it difficult to isolate an embryo-sac with the pollen-tube still in contact, the latter remaining in the micropylar region and in the apical

portion of the nucellus. In this species the wall is much thinner, and the granular remains of contents are not so apparent; the tubes are therefore more difficult to see except in section, and the usual twist formed on the entrance to the embryo-sac is replaced by a slight swelling of the tube (Fig. 9, *p. t.*).

Endosperm. After fertilization, as the oospore surrounds itself with a cell-wall, the definitive nucleus elongates and prepares to divide (Fig. 9, *d. n.*). This division, which never takes place before the first segmentation of the fertilized egg, is extremely rapid; amongst all the material examined not a single case of actual primary division was seen.

In preparations, however, showing the first division of the oospore, seven or eight endosperm-nuclei have been counted in a series of sections through the embryo-sac, and this was found to be the average number for this stage. These nuclei migrate at once to the periphery of the embryo-sac, where they lie embedded in the cytoplasm, merely dividing to keep pace with growth. At the micropylar and chalazal ends of the embryo-sac more rapid divisions occur, leading to aggregations of nuclei and a denser mass of cytoplasm at each extremity of the sac (Fig. 14, *end. c.*; Fig. 16, *e. s.*).

It has been shown (p. 32) that up to fertilization (Figs. 6 and 7) the uniform solution of the layers of nucellar tissue immediately in contact with the periphery of the embryo-sac points to its cytoplasm as being the digestive agency. This digestion after fertilization receives a definite impetus by the aggregation of endosperm-nuclei at the *antipodal* end of the sac (Fig. 16, *e. s.*), which elongates rapidly at the expense of the axile rows of the nucellar tissue situated immediately below the antipodals (Figs. 18, 19, *ax. c.*) until the wall of the embryo-sac arrives in proximity to the chalaza, where it enlarges somewhat (Fig. 19, *e. s.*).

A comparison of Fig. 7, in which the embryo-sac is fairly vertical and is exercising a destructive influence on the cells over its entire periphery, with Fig. 19, shows that some definite stimulus must have caused so distinct a line to be taken by one particular portion of an organ, and this fact is to be correlated with the aggregation of endosperm-nuclei at the chalazal end of the embryo-sac.

As will be shown later, there is reason to suppose that certain layers of cells at the base of the nucellus are specialized in a form which suggests a tissue for the passage of air and water.

Endosperm cap. Very active divisions of the endosperm-nuclei at the micropylar end of the embryo-sac result in an aggregation of nuclei, embedded in dense cytoplasm, in the vicinity of the basal suspensor cell, thus forming a cap surrounding it (Fig. 14, *end. c.*).

At about the time at which the cotyledons are first differentiated these endosperm-nuclei arrange themselves peripherally (Fig. 19, *end. c.*), free

cell formation subsequently follows, resulting in the formation of a single layer of small quadrate cells with dense protoplasmic contents (Figs. 20, 21, *end. c.*). This layer of cells keeps pace with growth by means of constant anticlinal divisions at the apex of the embryo-sac, but it gradually thins out into large uninucleated cells with vacuolated contents over the remaining portion (Fig. 21, *end.*). In the mature seed the endosperm-cap invests the root-cap of the embryo, and the rest of the embryo-sac is lined by a thin film of very large cells. The embryo is therefore enclosed by a single continuous peripheral layer of endosperm-cells of diverse character in the hypocotyledonary and cotyledonary regions respectively. In sections the cells composing the larger portion of the layer are difficult to see, owing to their size and extreme thinness, but they are easily differentiated by careful staining, and this portion can be dissected out in its entirety; but it is very difficult to get any part of the cap off on account of its intimate relation with the root-cap of the embryo on the one side and the nucellus on the other.

In consideration of these facts Hegelmaier (12) is hardly correct in describing the embryo as being enclosed in transitory endosperm in the Curvembryae, a group in which he had previously stated the endosperm to be limited entirely to the micropylar end (10).

Cell-wall formation in the endosperm is recorded for several families at a stage similar to that at which it occurs in *Stellaria media* of the Alsinoideae, that is, when the cotyledons first become differentiated. Guignard (17) records it for the endosperm of some Leguminosae, and concludes it becomes definitely organized at this stage to meet the increasing requirements of the embryo, the suspensor, it is assumed, being now no longer capable of doing so.

Strasburger (30), for the Eualchemillas, states that the endosperm forms walls as the embryo becomes heart-shaped. He makes the apposite suggestion that, as the embryo-sac is then full-sized, the stoppage of growth causes the endosperm-nuclei to remain in contact and so start cell division (30, p. 124). That this observation applies in the present case also is borne out by the regular arrangement of the nuclei of the endosperm-cap (Fig. 19, *end. c.*), which obtains just before cell division takes place. Péchoutre also (28) arrives at a similar result in the case of the Rosaceae.

In *Stellaria media* we see that the endosperm is differentiated in its apical portion into a compact layer of cells with dense and homogeneous contents, which in organization and appearance strongly suggest ferment-cells. This cap invests the apex of the embryo with its inner surface, whilst externally it is in direct contact with the axile rows of the nucellus (Fig. 22, *end. c.*). The cells of these rows adjoining the endosperm-cells show paucity of contents and very slight starch reaction, but they abut directly on the perisperm tissue of the nucellus, the latter appearing as

if it were constantly being drained of contents. There is no peripheral solution of tissue, as is the case in the vicinity of the basal suspensor cell, the endosperm layer merely consisting of passage cells. The entire absence of any other form of secretory tissue can only lead to the conclusion that the endosperm in this case is the medium through which the starch stored in the perisperm is made available for the growing embryo.

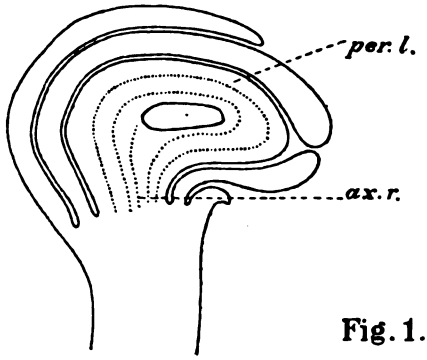


Fig. 1.

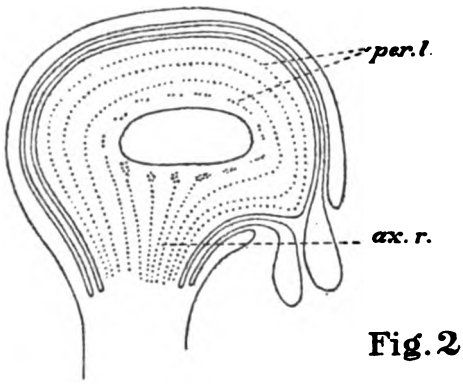


Fig. 2.

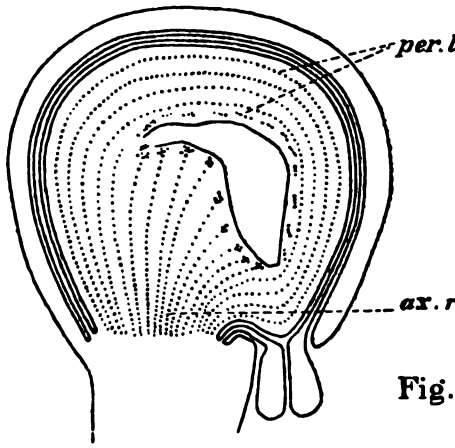


Fig. 3.

Figs. 1–3. The dotted lines represent the cell layers, and when interrupted show digestion of the same.

The Axile Cells of the Nucellus and the Perisperm. Before fertilization, continual vertical growth in the basal region of the nucellus results in an increase of the axile rows (*ax. r.* Text-figs. 1–3). Anticlinal cell division of the peripheral layers (*per. l.* Text-figs. 1–3), which from the earliest stages of development, is less active on one side than the other, now ceases altogether on the lower side, and the embryo-sac is thus forced from a horizontal to a more or less vertical position, by the campylotropous curvature of the nucellus characteristic of the order.

The axile cells are in serial connexion with the chalazal cells, and after fertilization two or three of the basal rows become vacuolated and the nuclei migrate to the walls, which cuticularize (*b. c.* Text-fig. 4, p. 38). These cells form a band across the chalaza connecting up on each side with

layer two of the inner integument (*i. i.* Text-fig. 4), the walls of the latter also becoming cuticularized (Pl. V, Fig. 18, *b. c.*). In a longitudinal section through the chalaza and base of nucellus (Fig. 12, *b. c.*) these cell layers are shown between the small polygonal cells of the chalaza and the rectangular layers of the nucellus. Their continuity with layer two, of the inner integument, can also be traced. The walls of these basal cells show pores, which do not occur on the cuticularized walls of the inner integument. These pores probably allow the free passage of water, for in these ovules the vascular tissue of the funicle does not penetrate beyond the chalaza (Figs. 18, 19, *v. b.*), and there is no vascular system in the nucellus or integuments. Nawaschin (22) figures a cuticularized area of thickened cells in the chalazal region of the elm ovule, which suggest comparison with the basal cells described above for the Alsinoideae. No further explanation is offered in the text.

Godfrin (15) notes that orthotropous and campylotropous ovules vary only in the nucellus being straight or curved. In both cases the hilum is directly under the nucellus and, with few exceptions, the seeds are non-vascular. Though he does not explain the fact, possibly the advantageous position of the chalaza, or hilum in the mature seed, has something to do with the efficient distribution of water, without the supplementary aid of a branching vascular system. The fact that as the ovules mature the terminal vessels of the funicle branch freely in the chalazal tissue may be adduced in support of this view (Figs. 18, 19, *v. b.*). The reserve food material is laid down directly over this the sole source of water supply, the cuticularization of the inner integument after fertilization, as described above, effectively cutting off all other channels.

In the mature seed and on germination large air spaces occur in the angles of the walls of the first two or three layers of the axile nucellar cells immediately above the cuticularized basal cells, which suggest the possibility of these latter cells forming a sort of aerenchyma (Pl. VI, Figs. 22 and 24, *a. s.*).

Perisperm. Before fertilization starch is limited to the layer of nucellar cells adjoining the embryo-sac (Pl. V, Figs. 6, 7, *st.*), the axile cells of the nucellus being as yet undifferentiated in respect of size and contents. After fertilization, however, starch is laid down very actively in those axile cells which immediately abut on the embryo-sac (*prm.* Text-fig. 4), and in this way the process of development of the perisperm is inaugurated in the nucellus (Pl. V, Figs. 16 and 18, *prm.*). Progressive development of the perisperm occurs (Pls. V and VI, Figs. 19, 22, *prm.*) until it forms a tongue of cells so densely packed with starch grains that the nuclei are squeezed out of all shape, but as the base of the nucellus, towards the chalaza, is approached, the starch contents become less and less, the cells are much smaller, the nuclei more and more active and the cytoplasm denser in

consistency, and quite at the base active cell division goes on, till maturity, when all the cells contain starch. This perisperm exercises a mechanical influence on the shape of the embryo-sac. The thin cytoplasm of the same has no effect on the dense contents of these cells (*prm.* Fig. 4). Careful investigation shows no sign of digested cells on the convex portion of the perisperm cells of the nucellus. Increase of breadth being thus effectually prevented on each side by the limits of the perisperm and testa respectively, the necessary expansion of the embryo-sac must take place in length and the natural effect of such a rigid mass of tissue is to increase the convexity of both embryo-sac and embryo. Hegelmaier (12) explains this development as a 'crescent-shaped portion of the nucellus which

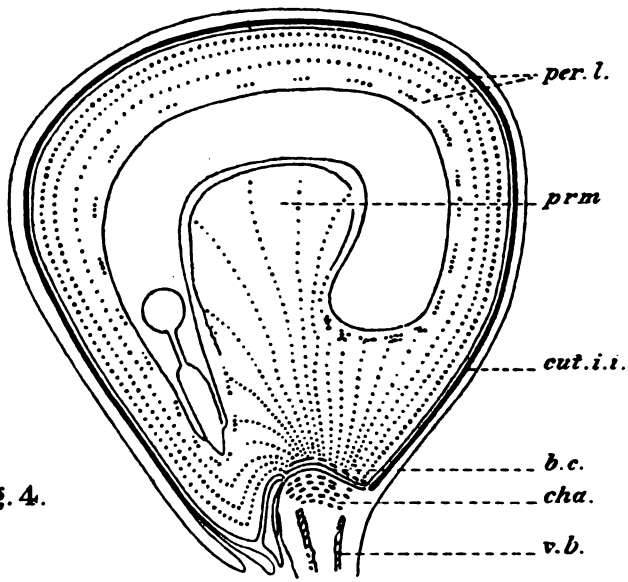


Fig. 4.

prepares itself for solution,' but that is perhaps rather an arbitrary method of description. Before fertilization, the whole nucellar tissue is homogenous. After fertilization, starch localization takes place, which, as we have seen, affects certain cells of definite layers ; but the fact that the layers digested during the growth in length of the embryo-sac are not specialized as storage tissue, hardly justifies one in saying they are ' prepared for solution.' Available for solution would be an expression more consistent with the facts, as the layers in question differ in no sense from the peripheral nucellar layers, which are constantly digested during the growth of the embryo-sac.

Peripheral layers of the nucellus. The peripheral layers of the nucellus are four to five cells thick before (Pl. V, Figs. 6 and 7, *per. l.*) and just after

fertilization (Fig. 16, *per. l.*, Fig. 18. *per. l*). The growth of the embryo-sac takes place at the expense of the inner peripheral layers, which are successively absorbed, and a disorganized row of cells always surrounds the embryo-sac with the exception of a small concave area near the chalaza where the perisperm is developing.

By constant periclinal and anticlinal divisions of the epidermal layer, the peripheral tissue keeps pace with the growth of the ovule, remaining about four or five layers thick. As the growth of the ovule becomes stationary, the meristematic activity of the epidermal layer relaxes (Pl. V, Fig. 19, *per. l.*) until in the mature seed (Pl. VI, Fig. 22, *per. l.*) the external layer alone survives. The cells of this layer increase in size and starch contents, but as their nuclei and cytoplasm remain active, they apparently retain the function of transitory starch storage tissue, which characterizes these peripheral layers from the fertilization stage. This epidermal layer in the seed persists till germination (Figs. 24 and 25, *per. l.*).

Suspensor. The suspensor is filamentous, consisting of one large cell, and succeeded by a varying number of smaller cells in vertical succession, never less than four, generally five or six (Pl. V, Figs. 14–18, *sus.*). The large basal cell (directed towards the micropyle) is formed immediately by the oospore, which elongates considerably after the fusion of the male and female nuclei (Fig. 9, *oosp.*). The resulting nucleus occupies the lower portion of the cell, surrounded by a dense reticulum. The upper portion then elongates, the cytoplasm becomes less dense, until in the extreme apex it completely loses its granular appearance and consists of a densely staining homogeneous substance (Fig. 9, *haus.*). The upper portion of the oospore in this plant elongates so much that it forms a haustorium at the micropylar end, which projects beyond the embryo-sac into the nucellar tissue. A certain compression is traceable in longitudinal section where the embryo-sac terminates (Pl. V, Figs. 9 and 15, *haus.*). In this tip the homogeneity of the contents remains distinct, and the wall is thicker in consistency.

The process is similar in other species of the Alsinoideae, in which the suspensor is not prolonged beyond the embryo-sac. The oospore enlarges and forms the basal suspensor cell, but the apex remains rounder, though it stains darker than the rest of the cytoplasm (Pl. V, Figs, 10, 11, *b. sus. c*). The enlargement of the oospore occurs very quickly. After the first division of the oospore to form the primary suspensor a large vacuole appears, at the end of the basal cell (Figs. 10, 11, 14, 18), and the nucleus stations itself just below it. This position is characteristic and persistent. The contents of the basal cell are extremely dense, the chromatin being lumped in the meshes of the reticulum. The nucleus is very large and active in appearance, and the cell suggests an absorbent organ.

After the first divisions of the primary suspensor the synergidae appear

contracted and empty and are not traceable further, owing to the rapid aggregation of nuclei and dense cytoplasm at this portion of the embryo-sac.

The fan-like arrangement of the cell rows at the apex of the nucellus indicates a convergence towards a given point (Figs. 16–18, *ap. nuc.*), and moreover these cells show disintegration in proximity to the base of the suspensor, and they are arranged with their long axes directed towards it. As the embryo grows the basal suspensor cell elongates (Pl. V, Fig. 19, *b. sus. c.*) and the contents become less dense and more granular, until finally, as the cotyledons develop and the organization of the endosperm cap follows, the suspensor is completely re-absorbed by the latter (Fig. 21, *dis. sus. c.*). The basal cell of the suspensor would thus form the first sucking organ, but, as the wants of the embryo increase, it is replaced by the endosperm cap, with its more complex organization and advantageous position, with regard to actual and potential food supply.

In the Alsinoideae a complete series is obtained in the grades of organization of the basal suspensor cell. In the Alsineae it reaches its greatest development, and in *Stellaria media* the climax may be said to occur. In the Sperguleae it is so reduced as to be hardly differentiated from the rest of the cells of the filament. The importance of this cell is indicated by its complex organization before the first division of the oospore (Fig. 9, *oosp.*).

Most work on the subject seems to point to the fact that the suspensor where it occurs is an absorbent organ. It may produce vermiform haustoria which seek available sources of food supply, as Treub (13) first described for orchids, or it may consist of large swollen cells charged with nutritive material, as in some Leguminosae (Guignard, 17). In the Alsineae the suspensor is very small if we exclude the first cell, consisting of only one row of three or four cells. In the Sperguleae it is more massive, and the cells divide again to form a double row; so that possibly the formation of the large basal cell, where it occurs, is to reinforce the absorbent power of the suspensor as a whole, just as the peculiar development of the micropylar and absorbent portion of this cell in *Stellaria media* suggests an attempt to increase the area of available food supply.

The inner integument. The integuments each consist, as has already been explained, of two layers of cells, forming four layers in all, of which three only persist in the ripe seed. These layers are at first undifferentiated in the case of each integument (Fig. 6, *o. i.*, *i. i.*). In the inner integument which arises first the cells at the apex increase in size as it closes over the nucellus, and these cells project far beyond the outer integument (Fig. 6, *i. i.*). Before fertilization they stain rather darkly (Fig. 7, *i. i.*), and after the passage of the pollen-tube they lose contents and shrink in size often leaving quite a cavity in which the tube persists. They

finally shrivel and close up (Fig. 18, *i. i.*) till in the mature seed the mycropyle is so pressed against the hilum that the outer integument practically closes over it (Pl. VI, Fig. 22). In the young ovule (Pl. V, Fig. 6, *i. i.*) the two layers are still distinct, the individual cells composing them showing centrally placed and active nuclei. As the ovule increases in size they become very much stretched (Fig. 7, *i. i.*), and after fertilization the dividing walls are more or less obliterated, the cells lose protoplasmic contents, and the nuclei disintegrate, the two layers practically fusing into one. ('Nährschicht' of Holfert (20).) The inner wall of layer two abutting on the nucellus assumes a wavy outline of highly refractive appearance. (Pls. V and VI, Figs. 21 and 22, *i. i.*) This inner wall immediately after fertilization becomes cuticularized in conjunction with some of the basal layers of the nucellus as already described on p. 36 (Fig. 18 *b. c.* and *i. i.*).

Outer integument. This consists also at first of two undifferentiated layers (Fig. 6, *o. i.*) but the cells of layer 1 soon increase in size and the nuclei drop to the base of the cell (Fig. 7, *o. i. i.*). After fertilization their walls begin to thicken and grow out but are not cuticularized until maturity (Fig. 19, *o. i.*). They finally form projecting papillae, the surface of the walls showing warty projections (Fig. 22, *o. i.* 1. *sec. pap.*) or small secondary papillae.

The second layer is composed of small cells with active centrally placed nuclei and denser contents (Fig. 7, *o. i.* 2). It suggests a transitory proteid layer and remains distinct till the embryo is well advanced (Fig. 21, *o. i.* 2), after which the contents gradually disintegrate, the cell-walls become crushed against the outer layer and apparently they merely increase the mechanical functions of the latter at maturity (Fig. 22, *o. i.* 2). ('Pigmentschicht' of Holfert (20).) The layers of the outer integument contain starch even in the germinating seed. Tannin is present in the cells of the integuments and the hilum.

This tannin seems characteristic of the moribund cells of the tegumentary layers which at maturation are purely protective.

Balfour (27) puts forward the suggestion that in non-vascular seeds the integuments form the water supply of the ovule. In the case of *Stellaria media* the only possible source of water supply is through the chalaza, the integuments being cut off at a very early stage by the cuticularization of the inside wall of the inner integument abutting on the nucellus. The base of the integuments, however, are in connexion with the chalaza (Pl. V, Fig. 12, *i. i.*), and the possibility of layer 2 of the outer integument forming a sort of water jacket to the growing ovule is suggested by the fact that in *Spergula arvensis* the wing which surrounds the ovule in the plane of the embryo is formed entirely from the proliferation of the cells of this layer (Pl. VI, Fig. 23 *o. i.* 2). These cells contain a little starch, their protoplasmic contents are not marked, and the nuclei are small. They

certainly do not form a proteid reserve, and they are too active in appearance not to suggest some necessary function. The proliferation occurs on the first divisions of the suspensor. On maturity the whole wing dries up forming merely a means for the dispersal of the seed. The differentiation of this wing at such an early stage would support the idea of transitory water storage, the later function to facilitate dispersal being only a secondary result of the transitory nature of the first.

If we take this view in the case of *Spergula arvensis*, there seems no reason not to apply it to the morphological representative of this tissue in *Stellaria media* where it is reduced to one layer which would thus form a specialized water jacket and not a transitory proteid reserve. This would bring the whole question into line with Balfour's apposite suggestion and seems to be the view borne out from the ontogenetic standpoint.

Chalaza. The chalaza is composed of small polygonal cells with large nuclei and dense homogeneous contents. These cells are in direct continuity with the nucellar axile cells and also with the layers of the integuments (Pl. V, Fig. 12, *cha.*). The vessels of the funicle abut on this tissue, branching as the ovule increases in size (Pl. V, Figs. 12, 18, 19, *v. b.*). In early stages it gives a xanthoproteic reaction. Before the funicle breaks off the cells become impregnated with tannin, and after rupture takes place it is bent up against the micropyle forming the hilum (Pl. VI, Figs. 22 and 24, *h.*).

Germination of the Seed.

Cerastium perfoliatum.

Germination begins by the elongation of the cotyledons into the central mass of perisperm, thus forming the first twist of a spiral (Pl. VI, Fig. 24). This elongation was observed in one or two cases in the mature seed, but is exceptional before actual germination, or hydrolysis of the starch reserves takes place.

In this stage the axile cells of the nucellus are elongated, and press laterally on the region between the hypocotyl and the cotyledons (Pl. VI, Fig. 24, *ax. c.*). They show a marked decrease in starch contents in the vicinity of the endosperm cap, the cells of which, on the axile side, practically form part of the nucellar tissue. The chalazal cells are almost obliterated, but the cuticularized layers of the 'aerenchyma' are apparent, large air spaces occurring in the nucellar cells immediately above them (Pl. VI, Fig. 24, *a. s.*). Transverse sections best show the intimate relation of the endosperm cap to the nucellus and root apex of the embryo. The activity of the endosperm is greatest in the first stage of germination, starch appearing in the epidermal cells of the embryo as soon as the growth of the cotyledons begins. A transverse section through the root cap shows the procambial strand (Fig. 25, *pc. s.*), the cortex, the outer layer of which

is densely filled with starch contents (Fig. 25, *c.*), the small cells of the root cap (Fig. 25, *r.c.*), succeeded by the enveloping layer of the endosperm cap cells (Fig. 25, *end. c.*) which present an actively secretory appearance and are connected as one tissue with the root of the embryo on one side, and the nucellus on the other. A section taken above the root cap still shows the endosperm cap cells (Fig. 26, *end. c.*), but in a section through the hypocotyledonary portion they are no longer seen (Pl. VI, Fig. 28).

The second stage in germination which occurs in a day or two according to the temperature and amount of moisture present, is marked by the apical portion of the endosperm being pushed slightly through the micropyle by the root apex on the elongation of the hypocotyl (Pl. VI, Figs. 31 and 32, *end. c.*). It is ruptured immediately (Fig. 33, *end. c.*) as the root grows through it, but the extruded portion which invests the hypocotyl as a collar persists on the seed coat after the cotyledons have been withdrawn (Fig. 35, *end. c.*). The basal portion remaining in the ovule is fused to the nucellus (Fig. 29, *end. c.*).

The cells of the ruptured endosperm cap lose contents and become vacuolated, they also elongate in a lateral direction (Pl. VI, Fig. 30, *end. c.*), but remain in connexion with the few strands of much compressed and empty axile cells which form the remains of the nucellus (Fig. 29, *end. c.* and *ax. c.*). The hypocotyl elongates with extraordinary rapidity and the development of root hairs being more or less simultaneous with the rupture of the endosperm cap (Figs. 33, 34, *r. h.*). In one case they were formed when the root was still in the micropyle. These facts seem to point to the conclusion that once water absorption can take place the embryo is independent of the ovule, though the whole cotyledonary portion may be still enclosed in the seed coat, for it can then utilize the starch which has been transferred to its tissues during germination.

The cotyledons have no connexion with the food reserves of the ovule. The epidermal layer is cuticularized on germination, when it reacts to iodine and sulphuric acid. Stomata appear on the dorsal and ventral surfaces as the hypocotyl emerges (Fig. 41). Air spaces occur in the mesophyll and a thread-like system of vascular strands pervades the lamelli of the cotyledons (Figs. 39 and 39 *a*, *tra.*).

These strands terminate below the apex of the lamelli in two pencil ends of tracheides which lie under a well-marked epithem composed of loose cells, large *water stomata* occurring in the epidermis on the dorsal side (Fig. 42, *w. sto.*).

In some cases where seeds were germinated in the dark with excess of moisture these stomata were more numerous, the epithem better developed, and the tracheids in greater number and more diffused.

The epithem region shows differentiation in the mature embryo, staining lighter than other portions. On germination it remains free of

starch, but the water stomata were not apparent till the formation of the root hairs, therefore not until the emergence of the hypocotyl, when root pressure would first be felt and some organization to regulate osmotic pressure must be called into play. The cotyledons are therefore perfect foliage leaves, the organization of which is complete long before they emerge from the seed coat.

The root apex of the embryo shows well-marked stratification in the mature seed with the root cap differentiated. Starch appears in the columella of the root cap after the differentiation of the root hairs (Fig. 34, *sth.*). This starch is localized to a few central cells of the columella and is evidently statolithic in function, as the grains are relatively few in number and only occur towards the base of the cells in normally growing seedlings.

Specific Differences.

Sperguleae. *Spergularia rubra* and *Spergula arvensis.*

Specific difference is rather marked in the Sperguleae, and runs in one or two lines more suggestive of a primitive form than variation from type.

In both *Spergula arvensis* and *Spergularia rubra* the nucellus is very much curved, bringing the micropyle almost in contact with the funicle, simulating an anatropous ovule. This peculiarity may be due to the greater packing of the ovules in the ovary, in *Spergularia rubra* especially they are extremely numerous.

The layer 2 of the inner integument shows an especial modification in *Spergula arvensis.* In the region of the endosperm cap and continued above it for a certain distance this layer consists of small and active-looking quadrate cells (Fig. 23, *i. i.* 2) which become strongly cuticularized, preserving their well-marked outline both in the mature and germinating seed (Pl. VI, Fig. 30, *i. i.* 2).

Layer 1 of the inner integument is indistinguishable. Over the rest of the ovule the inner integument behaves in the usual manner, viz. both layers fusing owing to the breaking down of the connecting walls through the extreme stretching they undergo to keep pace with the growing ovule (Figs. 23 and 29, *i. i.*). In *Spergula arvensis* layer 2 of the outer integument also undergoes modification, a local hypertrophy of tissue caused by the proliferation of the cells of this layer forming a wing all round the ovule in the vertical plane of the embryo (Fig. 23, *w.*). This wing is composed of small polygonal cells elongated in longitudinal section with very small nuclei and thin contents. As the ovule matures this tissue dries up, the empty cells remaining as an investing wing; it therefore serves a secondary function as a mechanism for wind dispersal. A water jacket may possibly be the first function of this proliferation of tissue. The appearance of the cells suggests water storage in the absence of dense staining contents which would characterize proteid preserves. They also

contain isolated starch grains which would not be traceable in cells containing proteid material. This wing is differentiated while the embryo is still in the suspensor stage.

The primary suspensor cell in both *Spergula arvensis* and *Spergularia rubra* shows a well-marked reduction in size. This is so apparent that Hegelmaier (10) speaks of the small celled suspensor of *Spergula arvensis*. Schleiden and Vogel (2) figure a row of undifferentiated cells of *Spergula pentandra*. This species was not available for examination in the present case, but in both the species investigated the primary cell is still considerably differentiated in size and contents from the succeeding ones of the row. The suspensor in the Sperguleae has a tendency to become more massive, as the cells composing it divide again vertically, thus forming a double row of cells. The primary cell does not divide again. The air spaces in the angles of the walls of the cells occurring just above the 'aerenchyma' layers are very pronounced in the mature and germinating seed of *Spergula arvensis*.

The synergidae are small and short in *Spergula arvensis* and long and narrow in *Spergularia rubra*.

Antipodals are well marked in both.

Alsineae. The synergidae fall into two types—

1. Long, with large nuclei, which attain their greatest development in *Arenaria trinervia* (Fig. 8, *syn.*) and *Stellaria media*.

2. And a shorter type with inconspicuous nuclei, which occurs in *Sagina procumbens*, *S. apetala*, and *Stellaria uliginosa*.

Antipodals are not always present or at least are not sufficiently obvious to be observed. They are well seen in *Sagina procumbens* and *S. apetala*, and in *Stellaria media*. In other species though clearly shown in the progressive free nuclear divisions of the embryo-sac nucleus, they were not so apparent at a later stage.

Definitive Nucleus. In all species, with the exception of *Stellaria media*, there is no actual contact between the nuclear membranes of the oosphere and definitive nucleus, some cytoplasm always separates them (Fig. 8, *oos.* and *d.n.*).

Basal suspensor cell. In *Cerastium* and *Stellaria* species this cell is very large. In *Sagina apetala* and *S. procumbens* it is smaller. It can be recognized in all the species before the division of the oospore, and the apex, though only prolonged as an haustorium in *Stellaria media*, shows the same marked differentiation in contents which stain darker and more homogeneously in that region.

Persistent pollen-tube. Is characteristic for all species except *Stellaria media*, and is especially well seen in *Sagina apetala* and *S. procumbens*, *Cerastium* species, and *Stellaria aquatica* (Fig. 13). The pollen-tube twists on itself before it enters the synergidae, forming a plug on the apex

of the embryo-sac, with which it is so intimately fused that it can always be dissected out still attached to the apex of the latter. The wall of the pollen-tube is very thick and the contents become granular. The tubes are well differentiated in Heidenhain's Iron Alum Haematoxylin.

Seed Coat. Shows distinct specific variation. The degree of cuticularization, the form and number of cells which enlarge and the degree of tannin formation seem to be constant characters. Yet even in this case variation is more apparent than real, depending chiefly on the papillose manner in which the cell-wall grows out, and the nature of the secondary projections which occur in it. In *Stellaria aquatica* the cells of the outer integument grow out broadly, using up their whole diameter. The ovule in consequence appears covered with papillae as the projecting cell-walls are almost in contact. In *Cerastium perfoliatum* only the immediate portion of the wall in the centre of a cell is raised, and radiating projections round the surface of the ovule result (Fig. 24, *o. i.* 1). The same remarks hold for *Spergularia salina* (Fig. 32, *pap.*). *Stellaria media* approximates more to *Stellaria aquatica* in the form of outgrowth of the individual cell-walls, but secondary warty projections occur in the wall of each cell (Fig. 22 *a*, *sec. pap.*). These projections in *Spergula arvensis* form large secondary papillae, one of which may grow out from each epidermal cell or in some cases only from a limited number of epidermal cells (Fig. 23, *sec. pap.*). In the so-called *id.* var. *sativa*, these projections are altogether absent, which, as the numbers are inconstant in the type, seems to hardly justify sub-specific distinction.

In both *Spergula arvensis* and its so-called var. *sativa* the wall has a wavy cuticle which in *Spergula arvensis* proper is continued in the same form on the secondary papillae (Pl. VI, Fig. 23, *pap.*).

Abnormalities.

In *Stellaria Holostea* a case of two megaspores in one ovule was seen (Pl. VI, Fig. 36, *m.*).

In *Cerastium glomeratum* two nucelli were observed in one ovule, each nucellus with a perfectly developed embryo-sac in the definite nucleus stage. As this abnormality has been fully described and figured in a previous paper (29) it is only referred to here.

In *Sagina procumbens* a very interesting case of vegetative outgrowth of the nucellus is figured on Pl. VI, Fig. 37. It occurs in a microtome series of the ovary and can be traced through four sections. No embryo-sac formation is apparent, the nucellus consisting of very actively dividing small cells, quite different in shape and contents from the tegumentary tissue of the ovule. It projects well beyond the integuments, which are not normally developed. In Fig. 37 the section is oblique, for while showing

the outgrowth of the nucellus best, it cuts into the cells of the outer integument on the posterior side of the ovule.

Conclusions.

The general result of work done on the Caryophyllaceae, section Alsinoideae, emphasizes the view that it is a very well-defined group of plants, the members being characterized by great uniformity both in the morphological development of the sporophyte and in histological structure. This uniformity extends to the reproductive organs, and investigation on the Alsinoideae in this direction tends to show that apart from more specific differentiation there is a certain developmental trend in the direction of greater specialization from the Sperguleae to the Alsineae.

If we pass in review the results obtained, the three most important points seem to be—1, the organization of the ovule in relation to the passage and storage of food supplies for the embryo; 2, the manner in which such food supplies are rendered available; and 3, the indication of certain lines along which development has proceeded within the group.

1. *The organization of the ovule.* The ovule in its complete form consists of the chalaza, a large nucellus with embryo-sac, invested by two integuments, and each of these component parts stands in important relation to the development of the whole.

The chalaza is the seat of elaboration of proteid material, and the whole of the organized food supplies required for the growth of the ovule and embryo, together with water and air, must pass through this tissue. It is situated in a very advantageous position, abutting on the vascular system of the funicle which branches freely into it during the later stages of development, whilst on the distal side its cells are in serial connexion with the axile rows of the nucellus. The perisperm is laid down in the upper region of these axile rows. Laterally, the chalaza is in communication with the integuments. The medium of diffusion between the chalaza and the nucellus would appear to be a few of the basal layers of the nucellus, the walls of which become cuticularized after fertilization and show shallow pits. In the mature seed and on germination large air spaces occur in the angles formed by the walls of several layers of the unmodified nucellar cells immediately above the cuticularized layers. This fact is suggestive of a possible function for these basal layers as a species of aerenchyma. That all gaseous interchange must necessarily be limited to this 'aerenchyma' is shown by the early cuticularization of the inner wall of layer 2 of the inner integument which effectually cuts off every other source of supply.

The immediate elongation of the embryo-sac which follows closely on fertilization, and its subsequent enlargement in the vicinity of the chalaza, is

also possibly correlated with the differentiation of the 'aerenchyma' in relation to the supply of water and oxygen.

We have seen that the integuments consist each of two layers which, in the case of the inner integument are undifferentiated, the cells of the apical portion merely increasing in size where they project beyond the outer integument. This part is subsequently used up during the passage of the pollen-tube. Over the periphery of the ovule they lose their cell contents, and become so stretched as growth goes on that the dividing walls disappear, leaving apparently one layer only.

The outer integument is composed of two differentiated layers, layer 1 being purely protective, increasing its area by the papillar outgrowth of the cells forming it and its mechanical function by the cuticularization of the cell-walls. It is possible that layer 2, the cells of which remain active and functional till maturity by dividing to keep pace with its growth, may act as a water jacket, forming a sort of transitory water storage tissue for the growing ovule. This hypothesis is strengthened by a comparison with the mode of its development in some of the Sperguleae. In these plants a proliferation of the cells of the layer under discussion results in a local hypertrophy, ultimately forming a wing which extends round the ovule in the vertical plane of the embryo, but in its earlier stages is very suggestive of a transitory water storage function.

The nucellus is differentiated into two regions, viz.: i. The peripheral layers, which are available for solution by the cytoplasm of the embryo-sac to provide for increase in size, and which when the latter obtains its maximum growth are gradually reduced to one layer, which persists till the germination of the seed and even in the discarded seed coat. ii. The axile rows which receive and distribute the supplies of food material from the chalaza in their basal portion and elaborate the starch reserves or perisperm in the upper cells of these rows, the cells increasing greatly in size as the starch is laid down.

2. *The manner in which the food supplies are made available.* That this occurs in the first place through the agency of the suspensor is suggested by the remarkable form assumed by the latter owing to the great differentiation of the basal cell of the filament. The early differentiation of that cell points to the same conclusion since it assumes its final shape even before the first division of the oospore (Pl. V, Fig. 9, *oosp.*).

The persistence of the pollen-tube, and the characteristic plug formed by it on the apex of the embryo-sac, may also be interpreted as corroborative evidence for the activity of the suspensor, as the channel formed by the pollen-tube in its passage through the nucellus is kept open, thus increasing the area available for solution. In *Stellaria media*, where the tube is not persistent and does not form a plug, the plant has overcome the difficulty in reaching the apical nucellar tissue by sending an haustorium

from the primary cell itself into this tissue. The function of the suspensor as a sucking organ would seem to be limited to the period preceding free-cell formation to the endosperm. As the basal cell elongates during the growth of the embryo the contents become less dense and more granular, and it finally remains as a mere empty sac.

The endosperm replaces the suspensor as the cotyledons are differentiated in the growing embryo, and we have seen that it is the apical portion alone which functions as a secretory organ, in the form of a cap composed of a single layer of cells, which invests the radicle. The endosperm is thus differentiated into two portions, structurally as well as functionally diverse—an active apical region, composed of small cells with dense contents, and an inactive peripheral portion with large and vacuolated cells which are stretched over the remaining surface of the embryo-sac. This differentiation is no doubt correlated with the favourable situation of the apical portion of the embryo-sac, being in immediate vicinity to the perisperm reserves of the nucellus and, through the lower axile cells of the latter, to the water supply through the chalaza.

As the seed matures, we see a further approach to these chief sources of food supply by the gradual pressing of the micropyle against the chalaza, which is characteristic of the maturation stage. In considering the autonomous organization for nutrition in these ovules, it is interesting to refer to the complex outside mechanisms in the case of *Phlox Drummondi* (Billings, 24), where in early stages the ovary wall is described as forming the starch reserve. A channel for the passage of food supply to the embryo is provided in the form of a papillose outgrowth of the ovary wall, in the vicinity of the micropyle; this papilla presses against the latter, which becomes closed and serves as conducting tissue.

The organization of the secretory cells of the endosperm in the Alsinoideae is very complete. They form an investing cap surrounding the radicle, which grows down into them, and completely fuse with the nucellus, forming an intimate connexion between it and the embryo which is only ruptured by the elongation of the hypocotyl on the germination of the seed. Even then their connexion with the nucellus is not affected, and they remain attached to the few strands of tissue which have not been absorbed by their agency for the benefit of the embryo sporophyte. In some chance sections through a seed, where a fungus mycelium had consumed the endosperm, evidently not being able to attack the perisperm, the embryo was malformed and undeveloped, with two straggling cotyledons composed of a few strands of tissue, the whole limited to the apical portion of the sac, thus pointing to the endosperm as the one agent for the supply of proper food and directive energy. Seeds from which the endosperm was artificially removed did not germinate. The results of the present investigation, as far as the Alsinoideae group of the Caryophyllaceae are

concerned, thus bear out Johnson's suggestion that the restriction in the formation of the endosperm to that of a purely digestive tissue which he observed in certain Piperaceae, obtains in all seeds with abundant perisperm, such as Chenopodiaceae, Polygonaceae, and Caryophyllaceae, but he goes on to say—

'Observations thus far lead me to believe that in the perisperm-containing seeds mentioned the embryo-sporophyte of the second generation is never nourished by the parent sporophyte directly, but always through the intermediate gametophyte.'

This view, as far as the Alsinoideae are concerned at least, only holds with regard to the ultimate organization of the embryo and the germination of the seed.

Before the cotyledons are differentiated everything points to the food material being digested and passed through the suspensor, and the embryo is accordingly nourished by the parent sporophyte up to that stage. It is only after the organization of the endosperm cap nuclei into a definite layer of cells that a portion of the endosperm takes on the function of secretory agent, which it retains till germination, when it is ruptured by the radicle on the elongation of the hypocotyl. A more limited function than is described by Johnson for the endosperm in some of the Piperaceae thus results from increased complexity and economy in organization; and the jacketing by the endosperm of the undifferentiated embryo at germination, which is such a striking feature in the Piperaceae, is reduced in the Caryophyllaceae to the short period necessary for the transference of the starch reserves in the perisperm to the tissues of the embryo on germination. The endosperm cells in this order fuse up more or less completely with the nucellus, and remain attached to the tissue of the latter when they lose connexion with the embryo, which is completely organized in the mature seed. There is certainly an elongation of the cotyledons in the seed prior to that of the hypocotyl, but the cotyledons in this stage are complete leaves, with a vascular system, cuticularized epidermis, stomata, and air spaces, and they are also provided with an epithem tissue and water stomata at their apices.

3. *Trend of Development.* In the Alsineae there seems to be a slight tendency towards greater specialization and development on the Spergulean type. The more massive and shorter suspensor occurring in the latter, with its small basal cell, is replaced by what may be a more labile filamentous one, in which the basal cell is greatly developed for absorption purposes, even to the producing of an haustorium as in *Stellaria media.*

If in the development of the integuments we look upon the wing which characterizes some of the Sperguleae and results from the local hypertrophy of cells of layer 2 of the outer integument, as primarily functioning as a water jacket, but subsequently becoming modified for wind dispersal on

the drying up of the cells composing it, we get a mechanism in which the latter function is often at a discount. In *Spergularia salina* winged and unwinged seeds occur in the same ovary (Pl. VI, Figs. 31, 34). In other species the same condition obtains, but more exceptionally. This fact suggests that water storage is the determining factor in the proliferation of this tissue, and where the supply of H_2O is deficient, or unequally distributed, the process of formation is interrupted. Therefore we might consider the local hypertrophy which is the origin of this band of tissue to be entirely suppressed in the Alsineae, and look upon the specialized layer of very active cells, capable by division of keeping pace with the growing ovule, and possibly of regulating water storage, as an advance in organization.

Finally, then, everything seems to point to the conclusion that the Alsinoideae are members of a very old and stable family. On one side they suggest an intermediate stage in the development of the ex-albuminous seed by a progressive reduction in the functions of the nucellus. The correlative increase in the activity of the endosperm results in the reserve food material being stored in the embryo itself through the medium of the latter tissue. M. Péchoutre's researches on the Rosaceae seem to point to that family as providing further illustration of the same tendency.

Among the points of comparison afforded by the Rosaceae may be mentioned the functional rôle played by the endosperm where some portion persists in the ripe seed.

The endosperm in this family is characterized by a limiting peripheral proteid layer (assize protéique), distinguished by abundant proteid reserves, but not otherwise differentiated from the other layers. In all cases some of this tissue persists in an active condition in the ripe seed. The conclusion drawn by Péchoutre that the function of this tissue is not mechanical, as in the case of the seed coat to which it is fused, but rather physiological in character, seems to be justified.

In the Alsinoidean stage of development the endosperm is limited to one layer only, and its function is entirely secretory and digestive. When this tissue increases in volume, the outer layer is specialized as a ferment layer, the increase in volume being associated with the increase in activity necessary to the transference of all the reserve food material through the endosperm to the embryo before germination, instead of its being stored in the nucellus to be drawn upon as required. If we consider the other end of the scale and take certain Piperaceae as a starting-point, a great restriction in the function of the endosperm is apparent in the Alsinoideae. In the Piperaceae the embryo at maturity is an undifferentiated mass of cells, and on germination the endosperm extrudes from the seed coat and jackets the embryo till cotyledons, hypocotyl, and root are organized.

In the Alsinoideae the endosperm has no other function beside that of secretion and digestion, and it does not bring these powers into play until

free cell division takes place. The complete organization of the embryo at maturity restricts the necessity for jacketing. It might perhaps be suggested that this restriction which obtains in the Alsinoideae is correlated with the more complete development of the suspensor as a primary digestive agent, and that this, by enabling the embryo to immediately draw on the organized food supplies of the parent, ensures the organization of the embryo being completed within the seed. The endosperm comes into play to supply what is beyond the capabilities of the suspensor as the embryo increases in size, and its function thus both begins before germination and continues afterwards on the same lines. As we get higher in the scale its activity after germination is more and more reduced until finally it is no longer present on maturity. The storage of the starch reserves in the embryo itself is another advance in specialization and economy. A small beginning is indicated by the starch which appears in the epidermal layer of the embryo in the Alsinoideae after germination, when the rupture of the endosperm necessitates the presence of some reserve to ensure continuance of growth till the cotyledons are drawn from the seed-coat and can assimilate on their own account.

In conclusion, I must thank the Curator of the Chelsea Physic Garden for supplying and growing material required for the purposes of this investigation; Mr. Malcolm Wilson, B.Sc., for very kindly collecting various species; and especially Professor Farmer for his unfailing kindness, help, and advice in the course of this work.

Bibliography.

1. Grew: Anatomy of Plants. 1682, Book IV, p. 196, Pl. 63.
2. Schleiden and Vogel: Ueber das Albumen. Act. Acad. Nat. Cur., xix, 1839, pp. 5–96, Pl. 41, Figs. 31–33.
3. Meyen, F. G. F.: Neues System der Pflanzenbiologie. Trans. Ann. Sci. Nat. Bot., xv, 2, 1841, p. 220, Pl. 16, Figs. 14–20.
4. Unger, F.: Anatomie und Physiologie der Pflanzen. 1855, p. 389.
5. Tulasne, L. R.: Nouvelles études d'embryogénie végétale. Ann. Sci. Nat., iv, 4, 1855, pp. 84–9, Pl. 14, Figs. 1–8.
6. Hofmeister, W.: Neuere Beobachtungen über Embryobildung der Phanerogamen. Pringsheim, Jahrb. wiss. Bot., i, 1858, pp. 82–188, Pls. 7–10.
7. Le Monnier, M. G.: Recherches sur la nervation de la graine. Ann. Sci. Nat. Bot., xvi, 5, 1872, pp. 233–304, Pls. 10–12.
8. Warming, E.: De l'ovule. Ann. Sci. Nat. Bot., vi, 5, 1877, pp. 177–266, Pls. 7–13.
9. Vesque, J.: Développement du sac embryonnaire des Phanérogames Angiospermes. Ann. Sci. Nat. Bot., vi, 6, 1878, p. 263, Pl. 11, Figs. 1–7.
10. Hegelmaier, E.: Vergleichende Untersuchungen über Embryoentwickelung dicotyledoner Keime. Stuttgart, 1878.
11. ———————: Untersuchungen über die Morphologie des Dicotyledonen-Endosperms. Nova Acta Leopoldina, 49, 1885, pp. 1–104, Pls. 5.

12. HEGELMAIER, E.: Ueber Orientirung des Keimes in Angiospermen. Bot. Zeit., 53, 1895, p. 163.
13. TREUB, M.: Notes sur l'embryogénie de quelques Orchidées. Naturk. Verh. Koninkl. Akad. Deel, xix, 1879, pp. 1–50, Pls. 1–8.
14. FISCHER, A.: Zur Kenntniss der Embryosackentwickelung einiger Angiospermen. Jenaische Zeitschrift Naturwiss., 14, 1880, pp. 90–132, Pls. 2–5.
15. GODFRIN, M. J.: Étude histologique sur les téguments séminaux des Angiospermes. Bull. Soc. Sci. Nancy, v, 2, 1880, pp. 109–219, Pls. 1–5.
16. WARD, H. MARSHALL: A contribution to our knowledge of the Embryo-sac in Angiosperms. Journ. Linn. Soc. Bot., xvii, 1880, pp. 519–46, Pls. 17–25.
17. GUIGNARD, L.: Note sur la structure et les fonctions du suspenseur embryonnaire chez quelques Légumineuses. Paris, Acad. Sci., Compt. Rend., 91, 1880, pp. 346–9.
18. ———— – : Recherches sur le sac embryonnaire des Phanérogames Angiospermes. Ann. Sci. Nat. Bot., xiii, 6, 1882, pp. 137–92, Pls. 3, 4.
19. LISTER, Miss G.: On the origin of the placentas in the Tribe Alsineae of the Order Caryophyllaceae. Linn. Soc. Journ. Bot., xx, 1883, pp. 423–9, Pls. 32–5.
20. HOLFERT, J.: Die Nährschicht der Samenschalen. Flora, lxxiii, 1890, pp. 279–313, Pls. 11–12.
21. SARGANT, E.: The formation of the sexual nuclei in *Lilium Martagon*. I. Oogenesis. Ann. Bot., x, 1896, pp. 445–7, Pl. 22–3.
22. NAWASCHIN, S.: Ueber das Verhalten des Pollenschlauches bei der Ulme. Bull. Acad. Imp. Sci. St. Pétersbourg, viii, 5, 1898, pp. 345–57, Pl. 1.
23. JOHNSON, D. S.: On the development of *Saururus cernuus*, L. Bot. Gaz., Sept., 1900, p. 208; Bull. Torr. Club, 27, 1900, pp. 365–72, Pl. 22.
24. BILLINGS, F. H.: Beiträge zur Kenntniss der Samenentwickelung. Flora, 88, 1901, pp. 253–318.
25. JOHNSON, D. S.: The Embryology and Germination of the Genus Peperomia. Abstract Science, 15, 1902, pp. 408–9.
26. ————: On the Development of certain Piperaceae. Bot. Gaz., xxxiv, 1902, pp. 321–40, Pls. 9–10.
27. BALFOUR, I. B.: The Angiosperms. Address to the Bot. Sect. Brit. Assoc. Adv. Sci., Glasgow, 1901.
28. PÉCHOUTRE, F.: Contribution à l'étude du développement des Rosacées. Ann. Sci. Bot., xvi, 8, 1902, pp. 1–158.
29. GIBBS, L. S.: Note on Floral Anomalies in species of Cerastium. New Phytologist, iii, 9 and 10, 1904, pp. 243–7, Figs. 81–4.
30. STRASBURGER, E.: Die Apogamie der Eualchemillen. Jahrb. wiss. Bot., xli, 1905, pp. 88–164, Pls. 1–4.

EXPLANATION OF PLATES V AND VI.

Illustrating Miss Gibbs's paper on the Seed of the Alsinoideae.

PLATE V.

Fig. 1. *Stellaria uliginosa*. Exceptional case of megaspore cutting off tapetal cell: *m.* megaspore; *t.* tapetal cell. × 1100.

Fig. 2. *Stellaria uliginosa*. Tapetal cell showing further anticlinal division: *m.* megaspore; *t.* tapetal cell; *ax. c.* axile cell-layer of nucellus. × 1100.

Fig. 3. *Arenaria trinervia*. Enlarging megaspore: *m.* megaspore; *ax. c.* axile cell-layer of nucellus; *ep.* epidermis. × 450.

Fig. 4. *Stellaria Holostea*. Epidermis showing increased anticlinal divisions at apex of nucellus: *m.* megaspore; *ax. c.* axile cell-layer of nucellus; *ap. nuc.* apex of nucellus. × 1100.

Fig. 5. *Cerastium glomeratum*. First division of embryo-sac nucleus: *e. s.* embryo-sac; *ax. c.* axile cells. × 1100.

Fig. 6. *Stellaria media*. Ovule with embryo-sac showing two polar nuclei: *e. s.* embryo-sac; *eos.* oosphere; *syn.* synergidae; *p. n.* polar nuclei; *ant.* antipodals; *ax. c.* axile cell-layers of

nucellus; *per. l.* peripheral layers of nucellus; *ap. nuc.* apex of nucellus; *st.* starch; *dis. c.* disorganized cells; *i. i.* inner integument; *o. i.* outer integument; *mic.* micropyle; *cha.* chalaza. × 400.

Fig. 7. *Stellaria media.* Ovule with embryo-sac showing polar nuclei fused into one definitive nucleus: *d. n.* definitive nucleus; *o. i.*[1], *o. i.*[2] layer 1 and layer 2 of outer integument; *i. i.*$_1$, *i. i.*$_2$ layer 1 and layer 2 of inner integument; other lettering as before. × 400.

Fig. 8. *Arenaria trinervia.* Embryo-sac showing oosphere, definitive nucleus and synergidae: *oos.* oosphere; *d. n.* definitive nucleus; *syn.* synergidae. × 450.

Fig. 9. *Stellaria media.* Oospore forming basal suspensor cell with haustorium. Definitive nucleus preparing to divide: *oosp.* oospore; *haus.* haustorium; *d. n.* definitive nucleus; *syn.* synergid; *p. t.* pollen-tube. × 1100.

Fig. 10. *Arenaria trinervia.* First segmentation of oospore into two suspensor cells: *b. sus. c.* basal suspensor cell; *end. n.* endosperm-nuclei. × 450.

Fig. 11. *Arenaria trinervia.* Suspensor with three cells: *b. sus. c.* basal suspensor cell; *end. n.* endosperm-nuclei; *syn.* synergidae. × 450.

Fig. 12. *Arenaria trinervia.* Longitudinal section through chalaza and nucellus, showing cuticularized basal cells connecting up with the chalaza and integuments: *nuc.* nucellus; *b. c.* basal cells of nucellus; *cha.* chalaza; *v. b.* vascular bundle of funicle. × 400.

Fig. 13. *Stellaria aquatica.* Apical cells of nucellus prolonged as papillae: *ap. nuc.* apical cells of nucellus; *nuc.* nucellus; *p. t.* pollen-tube; *b. sus. c.* basal suspensor cell. × 400.

Fig. 14. *Stellaria media.* Embryo-sac with embryo, showing aggregation of endosperm-nuclei at apical end (endosperm-nuclei on further side of embryo-sac shaded): *end. c.* endosperm cap; *b. sus. c.* basal suspensor cell; *emb.* embryo. × 450.

Fig. 15. *Stellaria media.* Next section in same series showing haustorium of basal suspensor cell protruding beyond embryo-sac: *haus.* haustorium; *b. sus. c.* basal suspensor cell; *e. s.* embryo-sac. × 450.

Fig. 16. *Cerastium glomeratum.* Longitudinal section through ovule: Embryo-sac elongating after fertilization: *per. l.* peripheral layers of nucellus; *b. c.* basal cells of nucellus; *e. s.* embryo-sac; *prm.* perisperm; *ax. c.* axile-cells. × 110.

Fig. 17. *Sagina apetala.* Longitudinal section of apex of ovule, showing persistent pollen-tube: *p. t.* pollen-tube; *p. t. t.* pollen-tube twist; other lettering as before. × 400.

Fig. 18. *Stellaria media.* Longitudinal section of ovule with embryo, showing perisperm formation in nucellus: *prm.* perisperm; *emb.* embryo; *sus.* suspensor; *fun.* funicle; *sus.* suspensor; other lettering as before. × 400.

Fig. 19. *Stellaria media.* Longitudinal section of ovule with heart-shaped embryo showing progressive perisperm formation in nucellus and reduction of peripheral layers: *cots.* cotyledons; other lettering as before. × 110.

Fig. 20. *Stellaria media.* Longitudinal section of ovule, showing embryo with cotyledons and endosperm cap; lettering as before. × 75.

Fig. 21. *Stellaria media.* Apical portion of same section under higher magnification, showing root apex of embryo, free-cell formation in endosperm cap and disorganizing suspensor; also cells of layer 1, outer integument, enlarging as papillae: *dis. sus.* disorganized suspensor; other lettering as before. × 110.

PLATE VI.

Fig. 22. *Stellaria media.* Longitudinal section of mature seed showing embryo, endosperm cap, one persistent peripheral layer and central perisperm mass of nucellus with cuticularized basal cells and air spaces above them: *pl.* plumule; *pc. s.* procambial strand; *sec. pap.* secondary papillae; *a. s.* air spaces; other lettering as before. × 110.

Fig. 22 *a.* Longitudinal section of the wall of a cell of layer 1 of the outer integument showing secondary papillae and wavy cuticle, after treatment with Eau de Javelle and I & H_2SO_4: *sec. pap.* secondary papillae; *cut.* cuticle. × 450.

Fig. 22 *b.* Surface view of wall treated in the same way: *sec. pap.* secondary papillae; *cut.* cuticle. × 450.

Fig. 22 *c.* Longitudinal section of basal cells of nucellus, showing shallow pits in the cuticularized walls: after treatment with Eau de Javelle and I & H_2SO_4. × 450.

Fig. 23. *Spergula arvensis.* Transverse section of ovule showing embryo-sac cut through the cotyledonary and hypo-cotyledonary portion, the proliferation of layer 2 of the outer integument, forming a wing of tissue round the ovule in the plane of the embryo, and secondary club-shaped papillae on the cell-walls of layer 1 of the outer integument: *w.* wing; other lettering as before. × 110.

Fig. 24. *Cerastium perfoliatum.* Longitudinal section of germinating seed, showing elongation of cotyledons; *h.* hilum; other lettering as before. × 110.

Fig. 25. *Cerastium perfoliatum.* Transverse section of germinating seed, same stage as Fig. 24, cut through the apex of embryo and nucellus in the root-cap region of the former, showing the sequence of tissues and their relation to the endosperm cap: Testa: *o. i.* outer integument; *i. i.* inner integument (two layers fused); *per. l.* peripheral layer of nucellus; *end. c.* endosperm cap; Embryo: *r. c.* root cap; *c.* cortical layers, outer densely packed with starch contents; *pc. s.* procambial stand. × 450.

Fig. 26. *Cerastium perfoliatum.* Transverse section of germinating seed; same series as Fig. 25, just above root cap; lettering as before. × 450.

Fig. 27. *Cerastium perfoliatum.* Diagram of transverse section of germinating seed showing the radicle of the embryo surrounded by the endosperm cap. × 110.

Fig. 28. *Cerastium perfoliatum.* Transverse section of germinating seed, same series, through hypocotyl of embryo, above the endosperm cap; lettering as before. × 450.

Fig. 29. *Spergula arvensis.* Longitudinal section through germinating seed, showing spiral elongation of cotyledons, extrusion of hypocotyl and subsequent rupture of endosperm cap, with axile cells of nucellus reduced to a few strands: *hyp.* hypocotyl; other lettering as before. × 75.

Fig. 30. *Spergula arvensis.* Same section, cells of endosperm cap under higher magnification, showing small quadrate cuticularized cells of layer 2 of the inner integument, which are only so modified in the apical region of the ovule in this species; lettering as before. × 450.

Figs. 31–34 are from seed which was three years old. Where the endosperm cap extruded slightly through the micropyle (Fig. 31, *end. c.*) the exposed cells had dried up and this portion formed a dark mark on the endosperm cap (Fig. 33, *end. c.*) which was observed on all these seeds.

Figs. 31–34. *Spergularia salina.* Germinating seed from the first extrusion to the rupture of the endosperm cap by the elongating radicle of the embryo to the formation of root hairs on the latter: *pap.* papillae (*formed by outgrowth of some cells of layer* 1, *outer integument*); *w.* wing; *end. c.* endosperm cap; *r. h.* root hairs; *r. c.* root cap; *sth.* statolithic starch; *hyp.* hypocotyl. × 75.

Fig. 35. *Spergula arvensis.* Seedling, cotyledons extruded, the endosperm cap remaining on the seed-coat: *cots.* cotyledons; *r. h.* root hairs; *end. c.* endosperm cap, mag.

Fig. 36. *Stellaria Holostea.* Longitudinal section showing two megaspores in one nucellus: *m.* megaspore; *ax. c.* axile cells; *epi.* epidermis; *ap. nuc.* apex of nucellus. × 1100.

Fig. 37. *Sagina procumbens.* Longitudinal section of ovule showing vegetative outgrowth of nucellus: *nuc.* nucellus.

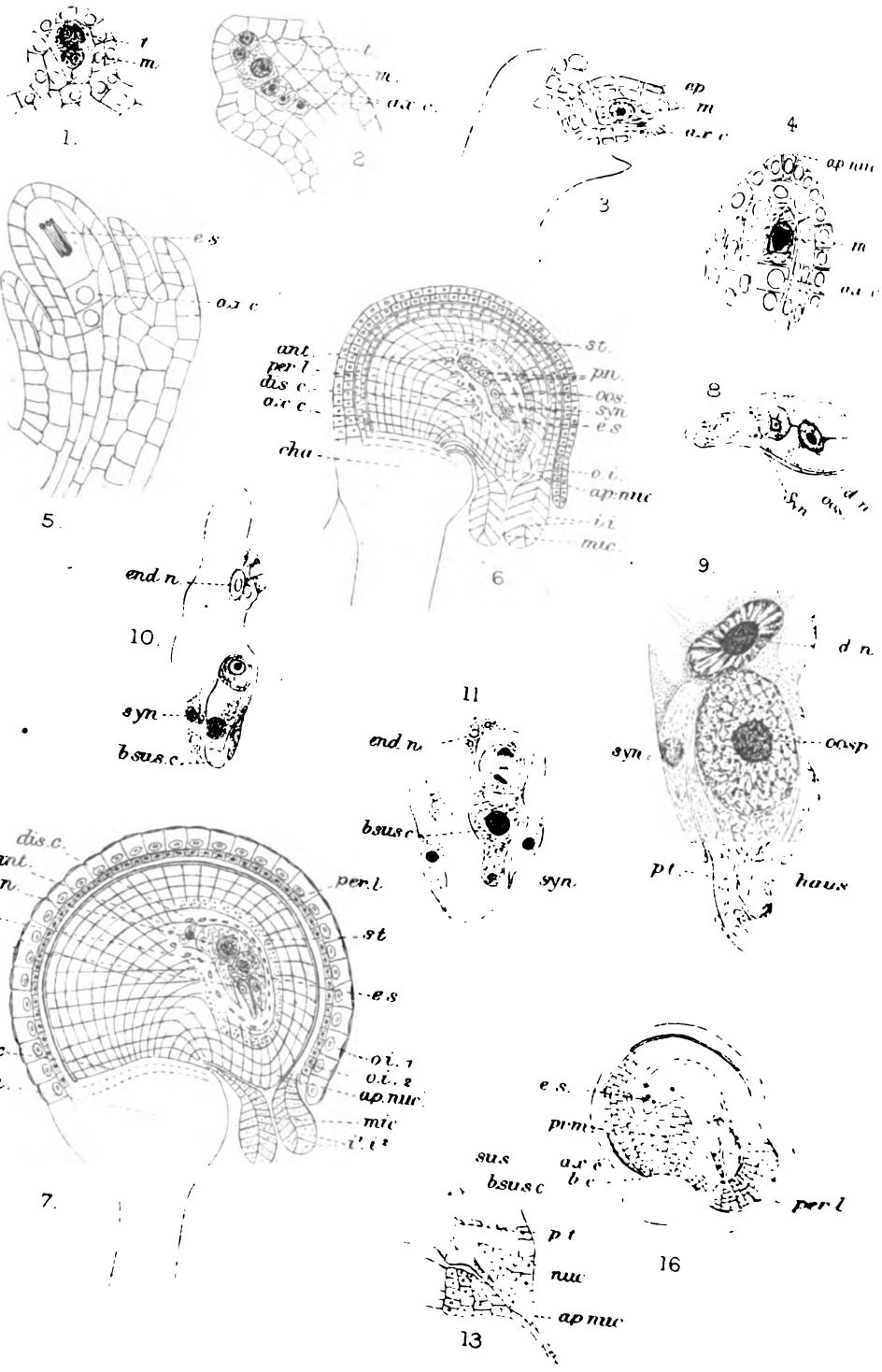
Fig. 38. *Sagina procumbens.* Apex of cotyledon just emerging from seed coat: *epi.* epidermis; *tra.* tracheids.

Fig. 38 *a.* Tracheids. × 450.

Fig. 38 *b.* Longitudinal section of cells of the epidermis with starch contents: *st.* starch; *cut.* cuticle. × 450.

Fig. 39. Surface view of stoma on ventral surface of cotyledons just emerged from seed coat: *sto.* stoma. × 450.

Fig. 40. Surface view of a water stoma surrounded by loose epithem cells, on dorsal surface of cotyledon, just emerged from seed coat. × 450.



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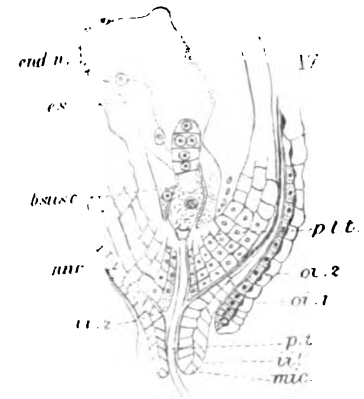

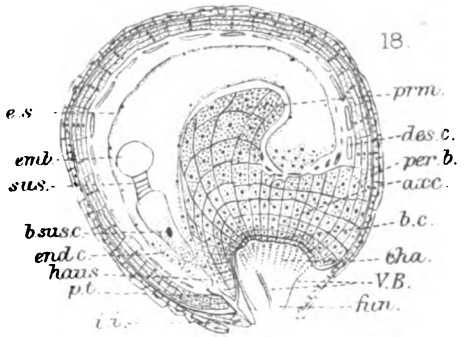

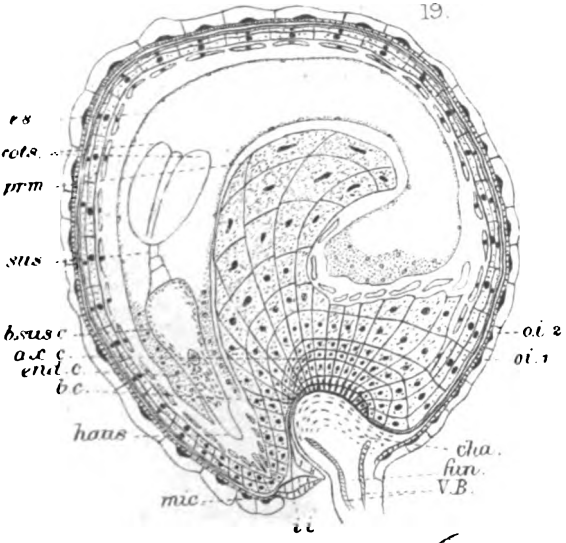

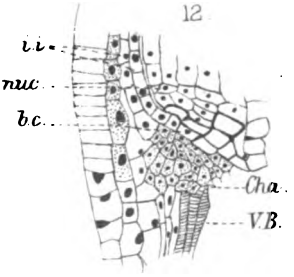

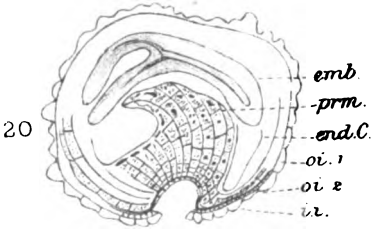

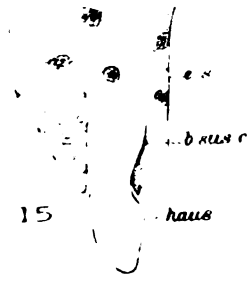

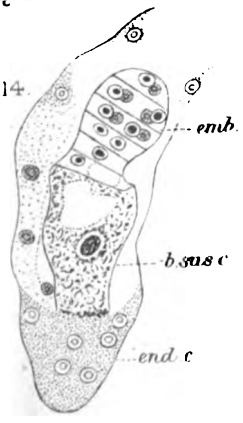

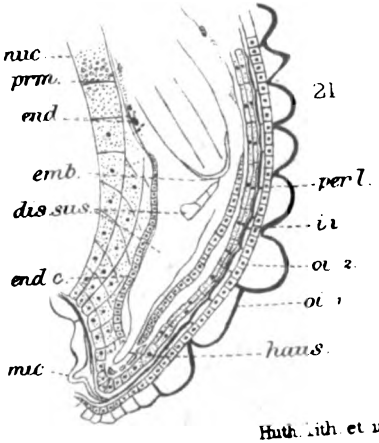

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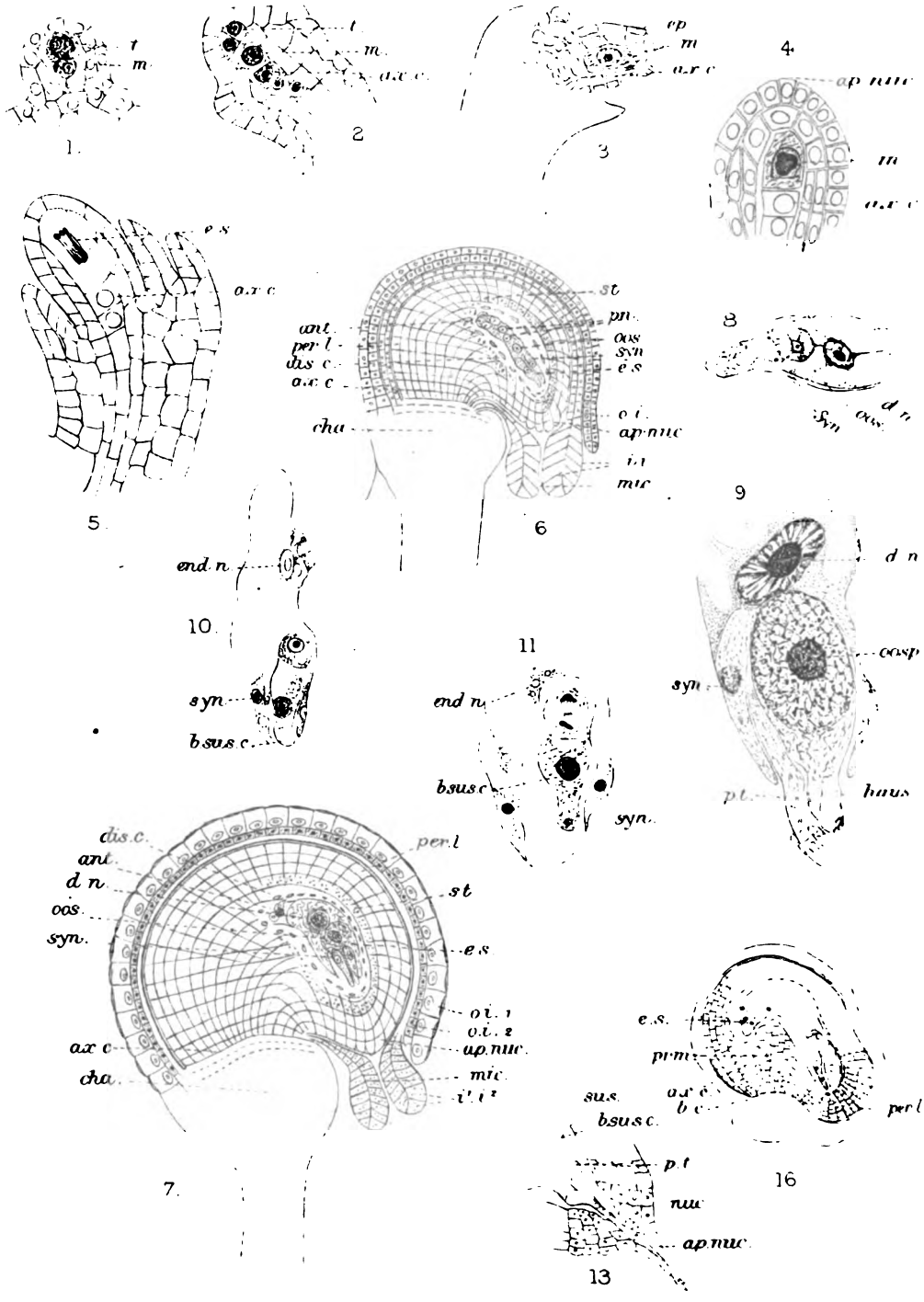

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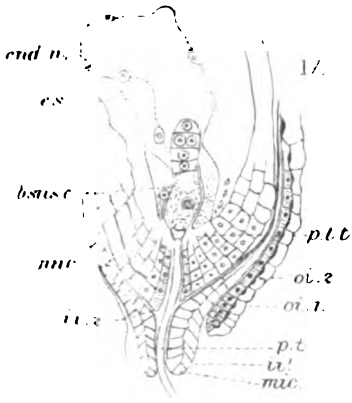

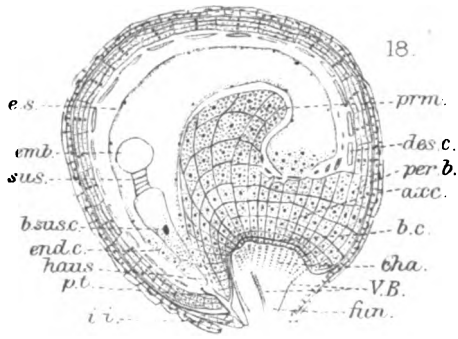

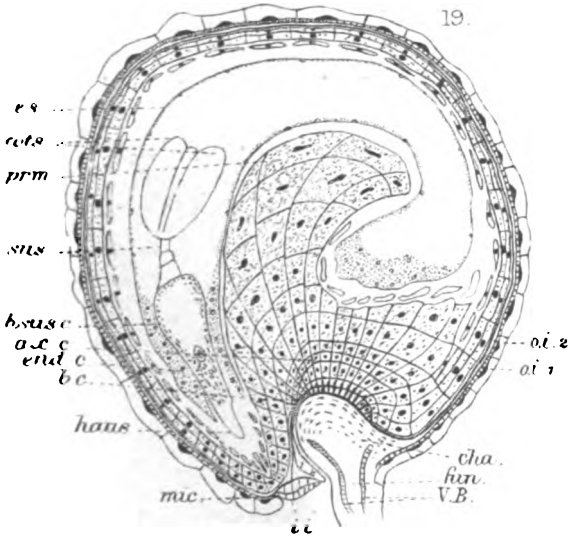

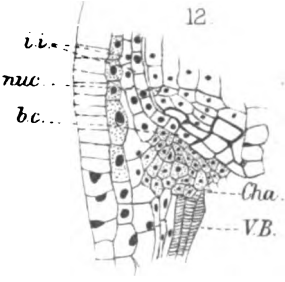

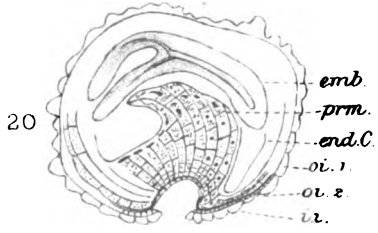

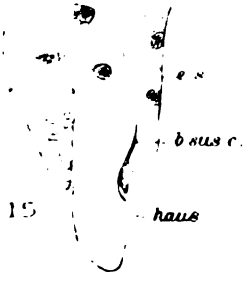

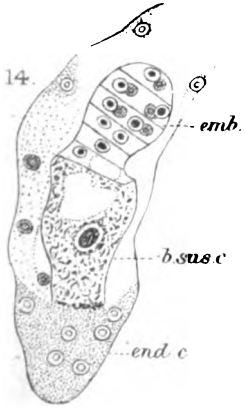

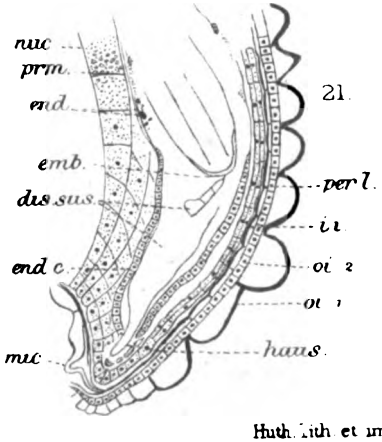

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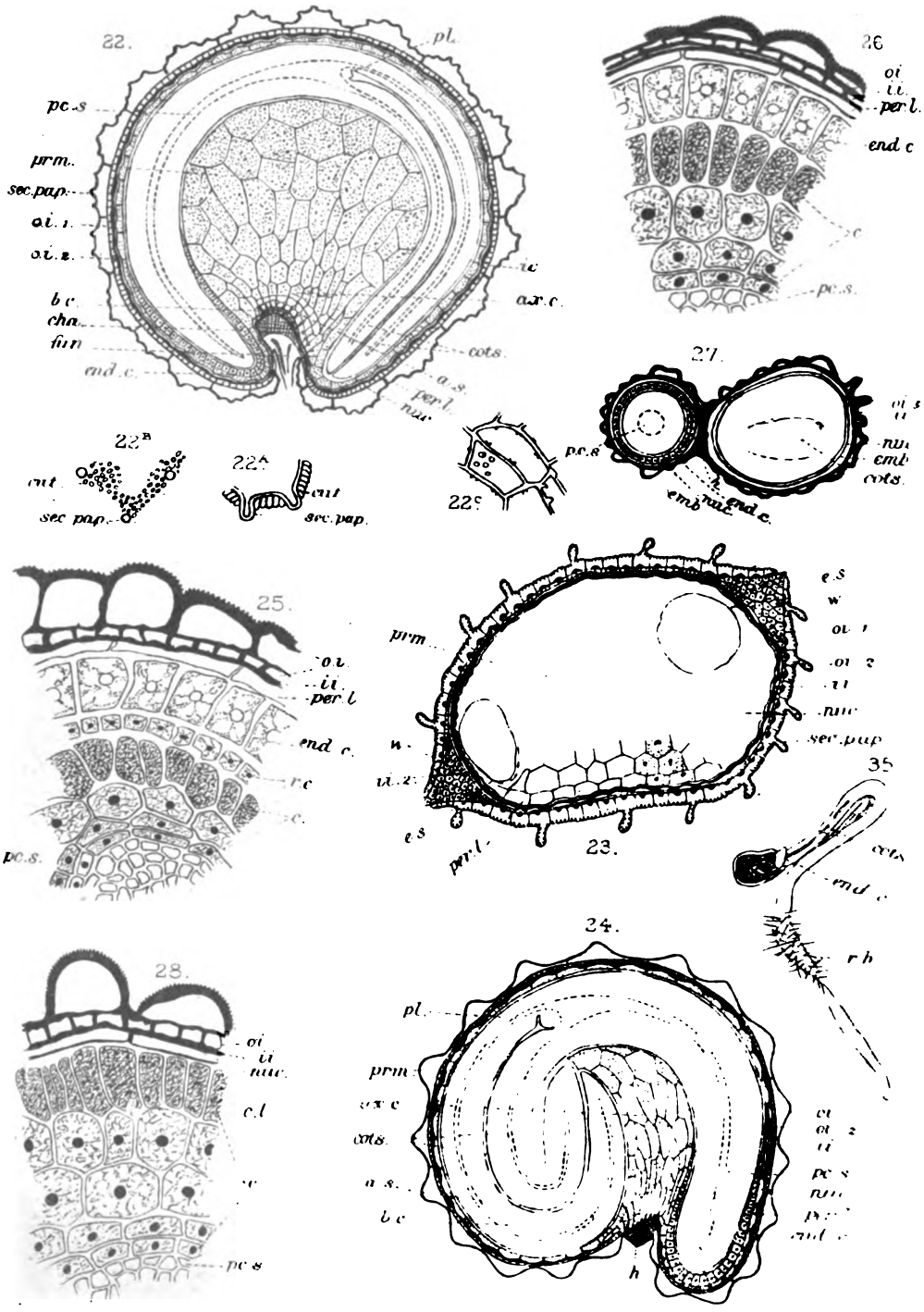
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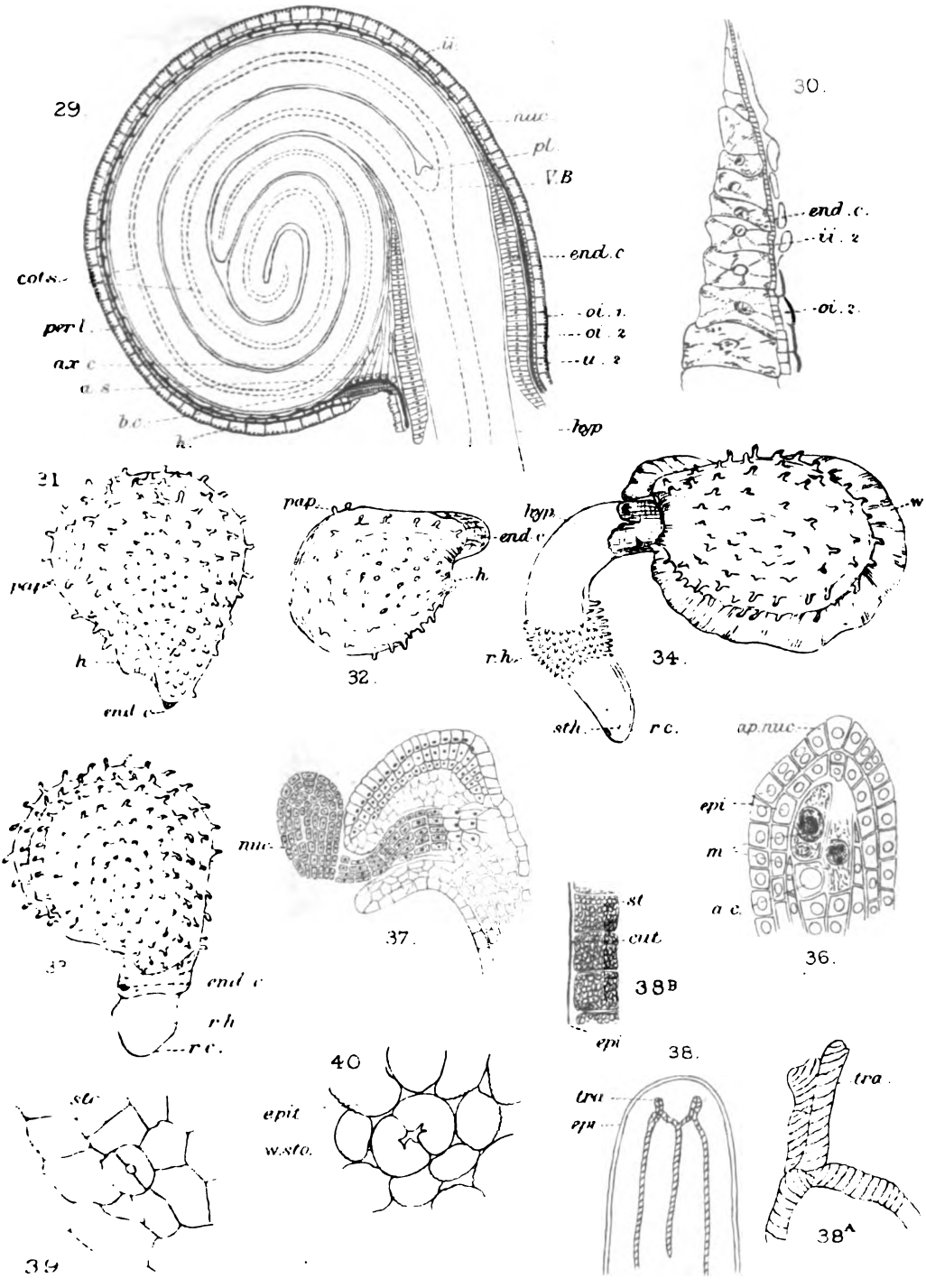


Huth, lith et imp.

On the Cause of 'Hardness' in the Seeds of Indigofera arrecta.

BY

C. BERGTHEIL AND D. L. DAY.

With Plate VII.

IT is well known that seeds possessed of hard coats, which do not allow of the penetration of water and consequent germination, frequently occur in certain leguminous plants, but no satisfactory explanation of the cause of this phenomenon has, so far as we are aware, been yet given. Attention has been drawn to the very marked case of hardness in the seeds of *Indigofera arrecta* by Leake (1), who points out that in the seed of this plant derived from Natal or grown in India the germination capacity normally varies from 5 to 10 per cent., but that by suitably scratching the seed-coat, in the manner commonly practised in the case of clover, it may be made to very nearly approach 100 per cent. These observations have been confirmed, and have been found to apply equally to seed derived from Java. The matter has assumed considerable industrial importance owing to the introduction of *Indigofera arrecta*, as a substitute for the previously grown *Indigofera sumatrana*, into the indigo districts of Bihar, and machines have been devised for scarifying the seed in bulk, in such a manner as to render its germination capacity satisfactory.

It has recently been found that the same end could be gained more simply, and with more certainty of success, by treating the seed with concentrated sulphuric acid; the seed resists the attack of this substance for twenty minutes to half an hour, and, after washing and drying, is found to germinate perfectly [1].

As a result of this observation it seemed to us of interest to try to determine to what cause the hardness of the seed was due, and how this cause was removed by scarification, and by treatment with acid. The only suggestion we have been able to trace as to the cause of hardness in seeds

[1] The idea of using sulphuric acid arose out of certain experiments carried out by Dr. Butler on the destruction of the eggs of insect pests in the lint of cotton-seed by treatment with this substance, after which, the germination capacity of the seed was found to be considerably improved.

[Annals of Botany, Vol. XXI. No. LXXXI. January, 1907.]

is made by Percival (2) to the effect that their testae contain an abnormally large amount of ash constituents, and that these resist the penetration of water. This explanation has been shown inadequate in the case under consideration by Leake (loc. cit.), who compared the amount of ash derived from testae of seeds of *Indigofera arrecta* with that obtained from the seed of *Indigofera sumatrana* (with the germination of which there is no difficulty) grown in the same locality, and found a considerably larger quantity in the latter case.

We have therefore sought for an explanation in the structure, or the nature, of the organic constituents of the seed-coat.

Preliminary examination of sections of the testa in an unstained condition, or treated with a simple undifferentiating stain (e. g. carmine or haematoxylin), showed its structure to be identical with that characteristic of the seeds of other leguminous plants. Superficially there is a layer of thick-walled 'palisade' cells with their longer axes parallel to the radii of the seed; beneath this is another layer of thick-walled cells of peculiar shape whose contents are pigmented yellow, and beneath this layer again, a double row of elongated cells with their longer axes parallel to the circumference of the seed; finally, there are several layers of ordinary parenchymatous tissues (Pl. VII, Fig. 1). No essential difference could be found in the structure of the testae of the seeds of *Indigofera arrecta* and *Indigofera sumatrana*, and in neither case could any traces of substances of a waxy nature be detected, either by examination of untreated sections or sections treated with osmic acid.

In order to ascertain how far water could penetrate into the hard seed-coat, sections were cut of seeds which had remained in water containing a little fuchsin for twelve hours without showing any signs of swelling. It was found that the stain had not penetrated beyond the outermost layer of the seed, and was deposited there in a sharp line (Fig. 1). On similarly examining seeds of *Indigofera sumatrana*, or those of *Indigofera arrecta* which had been scarified or previously treated with sulphuric acid, the stain was found to penetrate to the innermost layer of the testa (Fig. 2).

This seemed to clearly indicate that the resistant layer was to be found at the extreme outside of the hard seeds, and a careful examination was therefore made of the chemical nature of the substances composing the 'palisade' cells. On treating sections of such seeds with chlor-zinc-iodine (Schulze's solution), the cell-walls of all the tissues swelled and acquired the violet colour characteristic of cellulose, and the cell-contents the yellow colour indicating the presence of proteid substances. No cuticle could be detected by this treatment, and sections of the testa of *Indigofera sumatrana* seeds, and those of *Indigofera arrecta*, after treatment by scarifying or acid, presented an identical appearance to those of untreated

hard seeds. No explanation of the resistance to water in the latter case seemed, therefore, to be provided by this method. Sections treated with sulphuric acid and iodine lead to precisely similar results (Fig. 3).

Several attempts were made to demonstrate the existence of a cuticle in sections of hard seeds by methods of double staining (e. g. aniline-safranin counterstained with haematoxylin, and Gram-eosin), but in no case could a layer reacting towards these substances in the manner typical of cuticle be shown.

An explanation was eventually found by treating sections with a delicate stain for differentiating cellulose and cuticle described by Mangin (Zimmermann, 8), consisting of a solution of iodine in phosphoric acid. On treating sections of the testa of *Indigofera arrecta* seeds with this reagent, the cell-walls of all the tissues and the cell-contents reacted as cellulose and proteid matter respectively as before, but after a few minutes' action, a thin yellow line was seen to appear at the extreme periphery of the section, and to gradually swell as time progressed. On first appearing, this cuticular layer was only about 3–4 μ in thickness, but in the course of a quarter of an hour it had become more than three times this depth and was very clearly discernible (Fig. 4). Eventually the epidermal cells swelled enormously and ruptured, whilst the cuticle split away and was seen as a broad yellow band (Fig. 5). On treating sections of seeds of *Indigofera sumatrana* or of 'treated' *Indigofera arrecta* with this reagent no layer of this kind could be discerned. It was subsequently found that the cuticular layer characteristic of the seeds of *Indigofera arrecta* could be shown in sections treated with a concentrated alchoholic solution of chlorophyll, as recommended by Correns (4), but the test is difficult of application and uncertain in result.

It is clear, therefore, that the hard nature of the seeds of *Indigofera arrecta* is due to their possession of a very thin outer covering of a substance resistant to water. The exact nature of this substance cannot be determined by the methods which have been described. By its reaction under treatment with chlor-zinc-iodine, its swelling under the action of dehydrating agents, and its failure to respond to double staining methods, it would appear to consist of cellulose; whilst by its colour reaction towards Mangin's phosphoric acid and iodine, its staining with an alcoholic solution of chlorophyll, and its impermeability to water, it would seem to be cuticle. We can only conclude that it consists of a body of an intermediary nature, possibly a transition-product, between cellulose and cuticle.

The action of scarifying the seed is, no doubt, to remove a portion of this resistant covering, and so allow of the penetration of water, whilst sulphuric acid acts either in a similar manner by leading to the swelling of this layer and its eventual rupture, or by converting it into a body akin to cellulose and permeable to water.

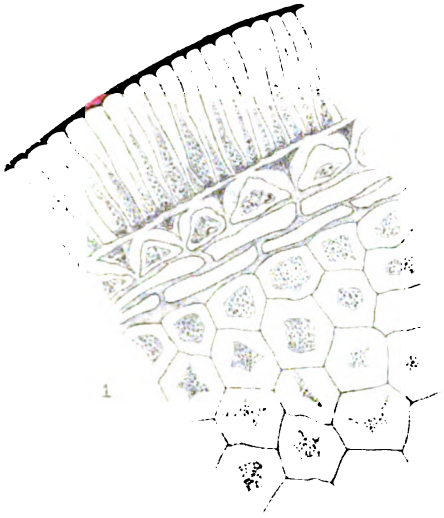
It would be of interest to determine whether this latter method of treatment would answer in other cases of hard seed, and whether their hardness can be traced to a similar cause to that which has been described.

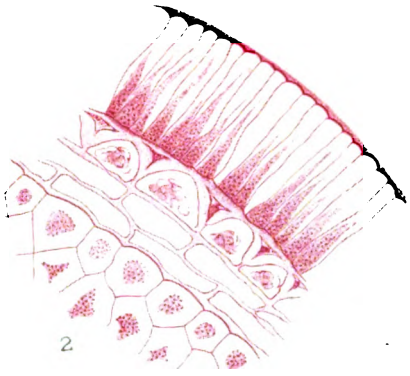
LITERATURE.

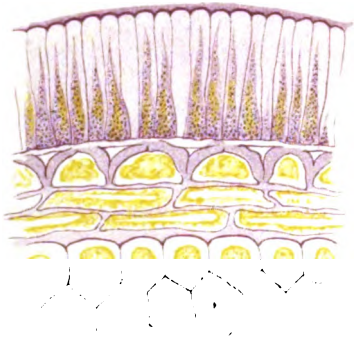
1. H. M. LEAKE: Journ. Royal Hort. Soc., vol. xxix.
2. J. PERCIVAL: Agricultural Botany, p. 623.
3. A. ZIMMERMANN: Die botanische Mikrotechnik, p. 138.
4. ————————: Ibid., p. 149.

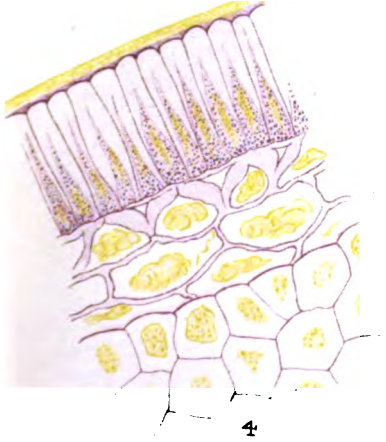
Since writing the above we have seen a paper by Jarzymowski (Inaugural-Dissertation, Halle, 1905), dealing with hardness in several leguminous seeds. This author holds that hardness is conditioned by the size of the lumina in the cells of the 'palisade' layer of the seed-coat. This explanation does not apply to the case of *Indigofera arrecta*. No difference can be detected between the shape or size of the lumina in such cells in the coat of this seed and in that of *Indigofera sumatrana*, nevertheless the former hardly germinates at all without treatment, and the latter germinates freely. The presence of the hard layer which has been described in the hard variety and its absence in the variety in which hard seeds do not occur, seems to point clearly to this being the determining cause in this case.

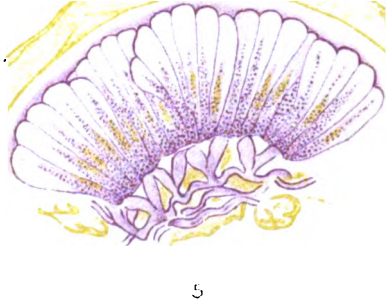
Jarzymowski has tested the method of treating hard seeds with sulphuric acid on several varieties and finds it to answer well; it appears to have been first suggested by Hiltner in 1902.











BERGTHEIL AND DAY —— INDIGOFERA.

The Bulbils and Pro-embryo of Lamprothamnus alopecuroides, A. Braun.

BY

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With Plate VIII.

LAMPROTHAMNUS is a genus of the Characeae placed in the subdivision Chareae on account of the presence of only five cells in the crown of the oospore: it differs from *Tolypellopsis* in the possession of stipular cells and from *Lychnothamnus* and *Chara* in having the oogonia below the antheridia.

Distribution. The plant has a wide distribution. It occurs in Europe in the countries of Scandinavia, Denmark, Germany, Spain, and England, though in the last-named country it is of very rare occurrence, having been found only in two localities, at Newtown in the Isle of Wight, from which locality it seems now to have disappeared (Groves, '90), and at the Fleet in Dorset (Mansell-Pleydell, '92). It occurs also in Africa (Braun, '67), but is not known in America, Asia, and Australia. The plants which I have investigated were grown from some dried mud sent from the neighbourhood of Port Elizabeth: they have now been growing in a healthy and apparently normal condition for more than two years.

General Features. *L. alopecuroides* roots in very fine mud or slime, growing to a height of about 15 cms. and branching very little. The internodes in the lower part of the plant may be as much as 4 or 5 cms. long, the leaves being 5 or 6 cms. The internodes decrease rapidly in length towards the apex of the plant, so that the whorls of leaves form a close tuft, which gives the plant that characteristic appearance whence it derives its specific name of *alopecuroides*. There is no formation of cortical tissue, and the whole plant has a rather delicate appearance. (See Pl. VIII, Fig. 1.)

The leaves are generally eight in a whorl, though on nearly every stem there are variations in one or two whorls, which may have seven, nine, or ten leaves, and with greater rarity either less or more than these numbers.

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The well-developed whorl of long, single-celled, pointed, stipular leaves or bracts occurring directly below the leaves and equal to them in number, is characteristic of the plant, as are also the leaflets springing from the axils of the leaves. Each leaflet has a sharp-pointed, colourless, transparent apex, into which the cell-lumen does not enter (Fig. 5 *a*). The stipular leaves measure 2 or 3 mms. in length and are here quite conspicuous, but the small leaves occurring in the axils of the ordinary leaves can only be seen on close examination. They resemble the stipular leaves in shape, but are much smaller and vary in length even in the same whorl: they are generally less than 1 mm. long. In the lower whorls fewer of these leaves are formed, there being two or three less in number than the leaves. In one case, taken at random, on the lowest nodes of a well-grown plant the number of small upper leaves formed varied from one to six in a whorl.

The development of the stipular leaves and of the axillary leaves has been studied by Giesenhagen ('02), who has shown that all three whorls, leaves, axillary leaves and stipular leaves, arise in a regular manner from cells which become divided into three by two horizontal walls. After regular subdivisions the upper cell gives rise either to an axillary branch or to an axillary leaf, the middle cell forms the foliar leaf and the lowermost cell the stipular leaf.

The plant is monoecious, the oospores, which ripen in autumn, standing singly below the antheridia which are of an orange-yellow colour.

Migula ('97) mentions that the plant may be slightly encrusted with calcium carbonate, but in the specimens on which I worked there was no encrustation except in the case of the oospores, which showed an accumulation of fine granules, but this never extended to the apex of the spore, so that to the naked eye the spores appeared greyish in colour, with a black apex where the hard, dark-coloured lamella was not hidden by the deposit.

The Bulbils. The plants under observation were grown in large glass jars, and being therefore under somewhat abnormal conditions, it is not extraordinary that the oospores were generally incapable of germination, though many seemed to arrive at maturity. The plants, however, showed great vegetative vigour, and reproduced abundantly from the characteristic unicellular bulbils which are produced on the roots. Similar unicellular bulbils occur in *Chara aspera*, in which species they have been described and figured by Giesenhagen ('02).

The tubercles or bulbils are about 1 mm. in diameter, and occur on the root node generally in groups of four or five, though larger groups are frequently found. They exhibit no rotation of protoplasm such as occurs in other internodal cells.

Mode of Growth of Tubercles. Tubercles may arise either directly by

the swelling up of one of the cells of a node bearing rhizoids, or from a rhizoid which has already grown out in the usual manner. In the former case, when the tubercle has attained its maximum size and has become filled with starch grains, one or more rhizoids may grow out from the distal or apical end. These rhizoids may immediately swell up to form new tubercles, which also become filled with starch, so that one or two new tubercles may be formed at the apex of the first tubercle.

The starch grains in these tubercles are smaller than in those first formed, indeed, a secondary tubercle appears always to remain considerably smaller than the first (Figs. 11, 12, and 14).

In a similar way the secondary tubercles may give rise to a tertiary tubercle, which in its turn remains smaller than the tubercle from which it springs.

The second way in which tubercles arise is as follows. In a rhizoid which has grown out from the node, is formed an oblique cell-wall, not far from this node (Fig 8 *a*). The cell so cut off begins to swell, and small starch grains appear (Fig. 8 *a*, *b*, *c*). At the node formed by the oblique wall, further division may occur into four cells which grow out into rhizoids in the manner usual in the Characeae: in these rhizoids, again, cell-walls may be cut off near the base to form secondary tubercles: tertiary tubercles are formed in the same way. Such sequences of two or three tubercles, though frequent, are not by any means usual: large numbers of tubercles may occur in which not one has grown out to form a secondary tubercle. At the base of primary tubercles there is always a small group of cells formed by further division of the four first-formed node-cells, as described by Giesenhagen in *Chara aspera*. There is no mention in this case of any occurrence of secondary tubercles. Their formation may be due, in the case of the *Lamprothamnus* under observation, to the formation of an unusual amount of starch in the plant, and the necessity of further provision for its storage.

Kuczewski ('06), writing on the subject of the multicellular bulbils of *Chara delicatula*, mentions that in his laboratory cultures of this species the root bulbils attained a remarkable size and were more plentiful than in nature. It would thus appear that in cultures the tendency is towards the greater development of this vegetative means of reproduction. Kuczewski also notes and figures a 'merkwürdige Erscheinung des Auftretens von Stärkekörnern in den langgestreckten Zellen der Rhizoiden.' In *L. alopecuroides* I have several times noticed the same appearance, but the starch grains in this case were not of the elongated form described by him in *C. delicatula*, but were rounded or somewhat angular, like those in the root tubercles. It is probable that they act as further supplies of reserve starch.

The rhizoids of *Lamprothamnus* have a single nucleus embedded in

the protoplasm a short distance from the apex, the apex itself being occupied by a number of small, round, shining bodies (Glanzkörper), which are constantly in oscillatory motion. The function of these Glanzkörper is disputed by Zacharias ('05) and Giesenhagen ('01), the latter considering them to be statolithic in function, the former holding that they have some function to perform in the thickening of the cell-walls of the rhizoid.

In these tubercles the nuclei are fragmented, a point which Giesenhagen failed to determine in the case of the tubercles of *C. aspera* owing to the difficulty of cutting sections, but it is probable from analogy that fragmentation is the condition there also. The nuclei can be best seen if tubercles are used from which the starch has been wholly or almost wholly withdrawn for the formation of young plants at the node. These tubercles should be fixed with chrom-acetic acid or Flemming's solution and stained with Delafield's haematoxylin or brazilin.

The nuclei resemble the fragmented nuclei of the internodal cells of the plant, but are somewhat more irregular in shape. They are much the same size, and divide similarly by the appearance of a constriction and the subsequent pinching off of a part of the nucleus.

The Pro-embryo. The pro-embryos arising from the rhizoid-nodes appear first as filamentous processes, at the end of which four horizontal walls are formed, thus dividing the filament into five cells, the three uppermost of which form the apex of the pro-embryo (Vorkeimspitze of Pringsheim). Two more walls then arise close to and parallel with the two lowest horizontal walls, thus cutting off two nodal cells; shortly afterwards three vertical walls are formed in the second nodal cell, and then peripheral cells are cut off. From these cells the first leaves are formed. From the cells of the first or rhizoid-node rhizoids are formed.

At this stage the pro-embryo has not appeared above the mud in which the plant grows and has formed no chlorophyll. I found no case in which the 'Vorkeimspitze' had more or less than three cells, though in *Chara* the number is four or five. In *Tolypella intricata* and some species of *Nitella* the number is two (De Bary, '75). The three cells differ in size: at first the apical cell grows until it is three or four times as long as the others; the protoplasm in it can be seen rotating, whilst the two lower cells are still filled with frothy protoplasm. With further growth the two lower cells become very much larger than the apical cell, which remains comparatively small. The middle cell elongates, and soon after the 'Vorkeimspitze' appears above the surface of the mud it begins to swell and becomes sometimes spherical, though generally remaining elongated (see Figs. 6 and 18). The lowest cell elongates greatly, and very often becomes turgescent and swells up, but it never becomes spherical as in the case of the middle cell. The cells which become spherical, or nearly so, are very easily

separable from the cells below, sometimes breaking off at a touch. The size attained by these cells of the 'Vorkeimspitze' is very striking when compared with the cells of the leaves (Figs. 1 and 5). The lowest cell sometimes attains a length of about 12 mm., whilst the middle cell reaches about half this length.

The first leaf-node becomes divided by three vertical walls into four cells, from three of which superficial cells are cut off and develop into leaves. The fourth cell is regarded as an apical cell, which by further division and growth forms the stem of the plant. The cells of the leaf-node are cut off before the cells of the rhizoid-node, hence the leaves begin to develop before the rhizoid cells.

From this first leaf-node (Uebergangsknoten, Pr.), which Pringsheim considers to consist of three imperfect nodes, each producing at most two leaves, arise the first true leaves (folia of Braun) of the young plant. The 'Vorkeimspitze' becomes pushed to one side by the growth of the leaves, so that in older plants it has very much the position of a leaf, from which it is nevertheless distinguishable here by its great size, the constant number of cells, and the fact that no leaflets (foliola of Braun) are formed, as in the case of the other leaves. Only four leaves of the 'Uebergangsknoten' or transitional node develop to any size, and of these the two leaves standing on each side of the apex of the pro-embryo are alike in size, and are larger than the other two leaves, which also resemble each other in size. The leaves of this whorl always remain uneven in length throughout the life of the plant. It can frequently be seen in older plants that in the two largest of the leaves of this node, the cell immediately below the uppermost cell becomes swollen, as in the corresponding cell of the 'Vorkeimspitze' (Fig. 1). There is a tendency, especially in the lower nodes of the plant, for the cells to swell in the same way, the wall becoming gradually thinner as the cell expands.

In one stem it was noticed that the first two nodes, which were separated by a long internode, bore leaves having swollen cells, whilst in the four succeeding nodes, which were close together, the leaves consisted of cells of normal size. In the nodes immediately above these, there again occurred long internodes and whorls of leaves having swollen cells. It is not uncommon for isolated leaves of a whorl, or even all the leaves of a single whorl, to show these swollen cells.

These large cells are good objects for observing the fragmentation of nuclei. As in other Characeae there is in each nodal cell throughout life, and in all young cells, a single large nucleus, spherical in shape and generally having a single large nucleolus, though several nucleoli may occur. This nucleus lies embedded in the frothy protoplasm filling the cell: division to form new cells takes place karyokinetically. In the young cell, when cell formation has ceased, elongation begins and a single large vacuole is formed

in the centre and the nucleus begins to fragment, so that in one of the large swollen cells there may be a hundred or more nuclei.

The fragmented nuclei of the large cells of *Lamprothamnus* resemble those described by Kaiser in *Chara foetida* (Kaiser, O., '96) to a great extent, but there appear to be a larger number which are elongated in shape than occur in the latter plant. The nuclei are varied and irregular in form: many are elongated, and divide by a gradual constriction of the middle portion of the nucleus (Fig. 17). In this figure of the nuclei of one of the swollen cells, various stages are seen: to the right is a nucleus which is just dividing across the middle, the two daughter-nuclei being connected only by a narrow neck. Other nuclei are rounded, or crescent-shaped.

Nordstedt, in referring to the pro-embryo arising from the spore, remarks that in *Lychnothamnus Wallrothii* (an old name for the plant) there occurs an embryo having an oblique node between the primary rhizoid-node (Samenknoten, or node at the apex of the spore from which rhizoids are formed) and the rhizoid-node or 'secondary rhizoid-node' of Nordstedt. Such an extra node I found in many of the pro-embryos formed at the root-nodes, and also in some produced from spores (Fig. 7). In the formation of this extra node an S-shaped wall is formed and a cell then cut off, which later divides into four, as in the node-formation in rhizoid structures (Fig. 7 A). By further division and growth rhizoids are formed, and these spread out in all directions, though they are actually formed in a tuft on one side only of the pro-embryo. This extra node varies in position: it is generally midway between the rhizoid-node proper and the point of origin of the pro-embryo, and is easily distinguishable from the rhizoid-node proper by the oblique wall. This extra or interposed node is by no means of constant occurrence: in the case of the pro-embryos arising from the underground nodes of the plant, taking nearly a hundred cases, I found that the proportion of plants having this extra node was one in three. In the case of the embryos grown from the spores, I was unable to determine the proportion, as the spores germinated so rarely. It is probable that this extra node is formed in embryos originating from a level at a somewhat greater distance below the surface of the mud than that from which the majority originate. The rhizoids of this interposed node would serve both to fix the young plant firmly and to provide it with nourishment, though probably the former function is the more important, as such large food supplies are available in the root tubercles. This hypothesis is borne out by the fact that if several pro-embryonic shoots arise from a single node, they are alike as regards the presence or absence of this extra node.

Pro-embryos may arise on underground nodes on which no root-tubercles occur, but, as is to be expected, the young plants generally arise in proximity to the stores of food material. Pro-embryos also very occasionally arise in the axils of the leaves.

Transitions from rhizoid to pro-embryo. There are sometimes transitions from a rhizoid formation to a pro-embryo (Nordstedt, '66). A long, thread-like rhizoid, divided in the characteristic way by oblique walls, may cut off cells at its apex to form the 'Vorkeimspitze,' leaf-node and rhizoid-node (Fig. 6) as already described. In such a case the extent of the pro-embryo cannot be defined, unless we consider it to be simply the internode below the rhizoid-node proper, in which the rhizoids are formed by the cutting off of peripheral cells, and all the cells above this rhizoid-node. Nevertheless, as there are so many cases in which an extra oblique node occurs below the rhizoid-node, when the extent of the pro-embryo is obvious, such a distinction is rather arbitrary.

With the further growth of the young plant of *Lamprothamnus*, as in the case of other Characeae, a branch, having a dome-shaped apical cell capable of continued division and the formation of whorls of leaves, is generally formed at the rhizoid-node proper (Fig. 1 *b*). This branch first grows out at right angles to the pro-embryo, but soon turns upwards to the surface of the mud. Occasionally an accessory pro-embryo arises from the rhizoid-node (Fig. 5).

Pro-embryo arising from the oospore. The few oospores which had germinated did so in late autumn. The pro-embryos formed, seemed weak and liable to the attacks of a fungus: they were all in a young stage, and the 'Vorkeimspitze' cells had not swollen. I found two cases in which an interposed oblique node was formed between the spore and the rhizoid-node (Fig. 7). The primary root, formed from the spore, divides by an oblique wall in the manner characteristic of the rhizoids of the group, and from the node so formed four rhizoids arise. Later, other rhizoids are formed from this node, and extra rhizoids arise from the 'Samenknoten' (Pr.) or primary rhizoid-node (Nordstedt) situated at the apex of the spore.

Abnormal Plants. (1) Springing from a root-node on which were two tubercles, I found a young pro-embryonic plant, which, instead of showing the usual rhizoids springing from the rhizoid-node, showed about nine rounded tubercles of various sizes, evidently formed directly from the node-cells. Not a single cell had grown out in the ordinary way to form a rhizoid. Between the rhizoid-node and the node from which the pro-embryo sprang, rows of small starch grains were deposited. The grains in the different tubercles were of different sizes, varying directly with the size of the tubercle itself.

(2) Another abnormal case occurred in which at a rhizoid-node showing the S-shaped oblique wall, there arose, together with one or two rhizoids, a rounded tubercle filled with starch grains. The tubercle had formed chlorophyll and was of a bright green colour. Arising from the same node and in close proximity to the tubercle was a single leaf segment made up of about five cells. It would seem that this part of

the plant must have been exposed to the light, and so had become green. With a view of confirming this I exposed tubercles to the light at the surface of the mud, but in no case could I obtain the formation of chlorophyll.

Summary of Results.

Under cultivation in jars in which there was no special provision for aeration, but in which the water was from time to time changed, *L. alopecuroides* has flourished vegetatively for several years, showing its characteristic growth and producing an abundant supply of both antheridia and oogonia.

Apparently a very small number of spores are capable of germination, producing a pro-embryo of characteristic growth.

The pro-embryos produced from the oospores resemble those produced from the underground nodes of the plant.

In many cases the pro-embryo differs from that of other Characeae by the interposition of an extra oblique node, from which rhizoids are produced.

In the case of the pro-embryos produced from the rhizoid-nodes bearing tubercles, about 30 per cent. showed this interposed node.

For the most part reproduction takes place by means of pro-embryos, which are formed on the rhizoid-nodes and make use of the starch stored up in the tubercles. Branch pro-embryos are rare.

Sometimes pro-embryos arise from rhizoid-nodes bearing no tubercles, or from the rhizoid-node of another pro-embryo.

The tubercles either originate directly as such, or are formed by the transformation of rhizoids.

The terminal rhizoid of a tubercle may again become transformed into a tubercle containing starch, thus forming a series of two or more tubercles.

The pro-embryos arise at the basal side of the tubercle.

Generally several pro-embryos arise from a node bearing tubercles.

The nuclei of the tubercles are fragmented, as in the case of the internodal cells.

In concluding, I desire to express my sincere thanks to Professor F. E. Weiss, who suggested this piece of work, and whose interest and help have throughout been most valuable to me.

INDEX OF LITERATURE.

DE BARY ('75): Zur Keimungsgeschichte der Charen. Bot. Zeitung, vol. xxxiii, 1875, trans. in Journal of Botany, 1875.

BRAUN, A. ('52): Ueber die Richtungsverhältnisse der Saftströme in den Zellen der Charen. Ber. d. kgl. Akad. d. Wiss. zu Berl., 1852 and 1853.

——— ('67): Die Characeen Afrikas. Ber. d. kgl. Akad. d. Wiss. zu Berl., 1867.

BRAUN AND NORDSTEDT ('82): Fragmente einer Monographie der Characeen. Ber. d. kgl. Akad. d. Wiss. zu Berl., 1882.

DERSKI ('97): Beobachtungen über Kerntheilung bei *Chara fragilis*. Jahrb. für wiss. Bot., 1897.

———8): Weitere Beobachtungen an *Chara fragilis*, *t. c.* 1898.

GIESENHAGEN ('01): Ueber innere Vorgänge bei der geotropischen Krümmung der Wurzeln der Charen. Ber. der deutsch. bot. Gesell., Band xix.

——— ('02): Untersuchungen über die Characeen. (I. Die Wurzelknöllchen der Characeen. Flora, Bd. 82, Jahrg. 1896. II. Der Bau der Sprossknoten bei den Characeen. Flora, Bd. 83, 1897, and Bd. 85, 1898.)

GROVES, H. AND J. ('90): Notes on the British Characeae. Journ. of Bot., 1890.

KAISER, O. ('96): Ueber Kerntheilung der Characeen. Bot. Zeitung, 1896.

KUCZEWSKI, O. ('06): Morphologische u. biologische Untersuchungen an *Chara delicatula* f. *bulbillifera*. Beihefte zum bot. Centralblatt, 1906.

MANSELL-PLEYDELL ('92): On the occurrence of *Lamprothamnus alopecuroides* in Dorsetshire.

MIGULA ('97): Die Characeen. Rabenhorst's Kryptogamenflora, 1897.

NORDSTEDT, O. ('66): Några iakttagelser öfver Characeernas gröning. Lunds Univers. Årsskrift I. II.

PRINGSHEIM ('62): Ueber die Vorkeime der Charen. Monatsb. d. kgl. Akad. d. Wiss. zu Berlin, 1862.

——— ('63): Ueber die Vorkeime u. die nacktfüssigen Zweige der Charen. Jahrb. für wiss. Bot., vol. iii, 1863.

VINES ('78): Pro-embryo of *Chara*. Journ. of Bot., 1878.

WAHLSTEDT U. NORDSTEDT ('75): Ueber die Keimung der Characeen. Flora, 1875.

ZACHARIAS ('05): Ueber Statolithen bei *Chara*. Ber. der deutsch. bot. Ges., 1905.

EXPLANATION OF PLATE VIII.

Illustrating Miss M'Nicol's paper on *Lamprothamnus*.

pa. pro-embryonic apex; *ln.* leaf-node; *r. n.* rhizoid-node; *i. r. n.* interposed oblique rhizoid-node; *r.* rhizoid; *b.* branch with dome-shaped apical cell.

Fig. 1. *Lamprothamnus alopecuroides*. Natural size.

Figs. 2, 3, 4. Successive stages in growth of pro-embryo.

Fig. 5. Young plant from the rhizoid-node of which an accessory pro-embryo has arisen.

Fig. 5 A. Characteristic sharp apex of leaflet.

Fig. 6. Transition from rhizoid to pro-embryo.

Fig. 7. Pro-embryo arising from an oospore, and showing an interposed root-node.

Fig. 7 A. Node of rhizoid showing characteristic formation of four cells. This figure has been accidentally inverted in lithographing.

Fig. 8 *a*, *b*, *c*. Stages in the formation of a root tubercle. In *a* starch is beginning to be formed. In the other tubercles the presence of starch is indicated by a wash.

Fig. 9. Fully formed tubercle.

Figs. 10, 11. Beginning of formation of secondary tubercle.

Fig. 12. Fully formed secondary tubercles.

Fig. 13. Case in which the primary tubercle has remained elongated whilst the secondary tubercles have become spherical.

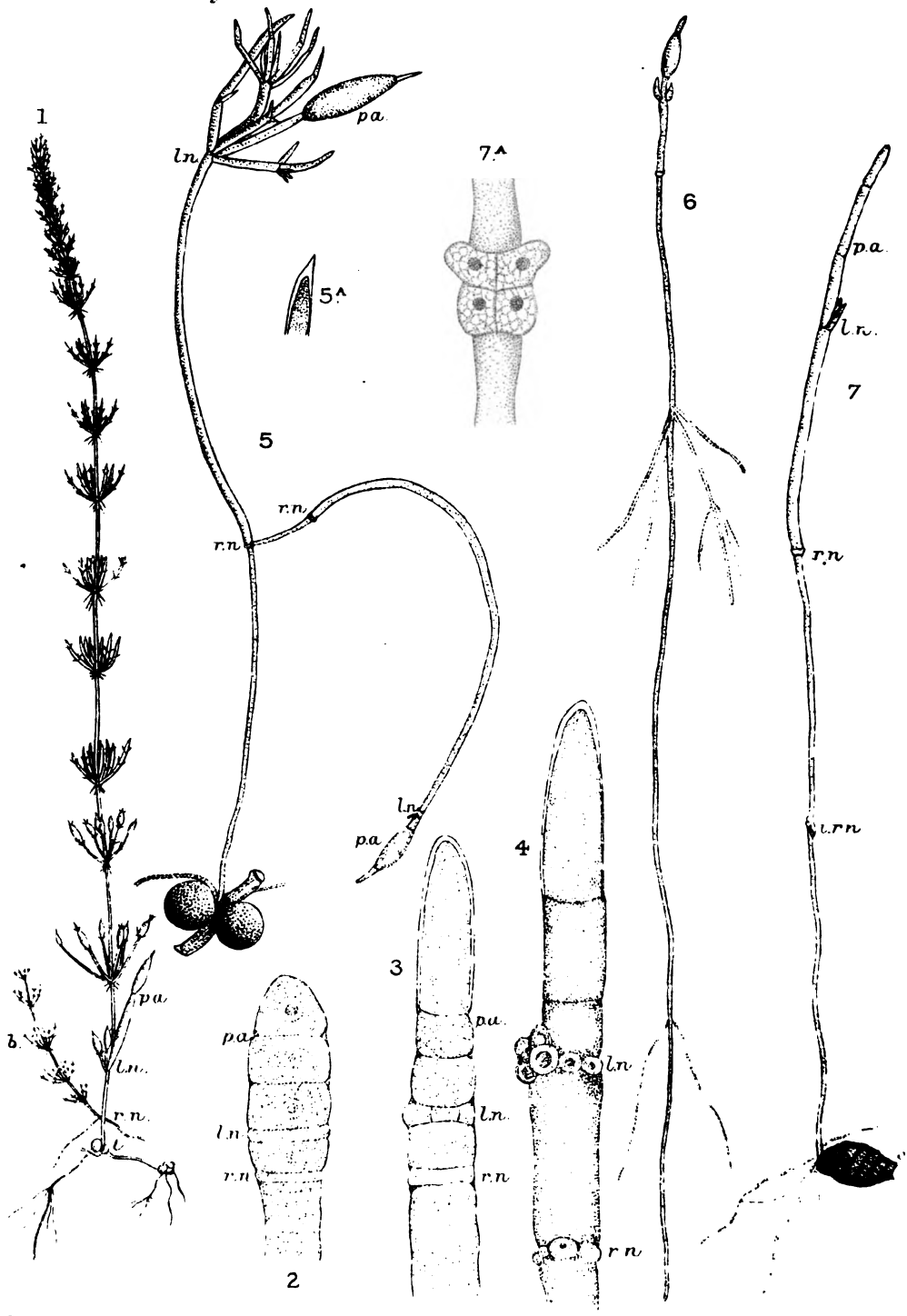
Fig. 14. Sequence of 3 tubercles.

Fig. 15. Group of tubercles showing primary, secondary, and tertiary tubercles in process of formation.

Fig. 16. Fragmented nuclei of a root tubercle, *s*. Starch grain.

Fig. 17. Fragmented nuclei of one of the swollen cells of the pro-embryonic apex.

Fig. 18. Pro-embryo formed from tubercle near the surface of the mud, and consequently shortened in growth. A young pro-embryo is also seen to the right.



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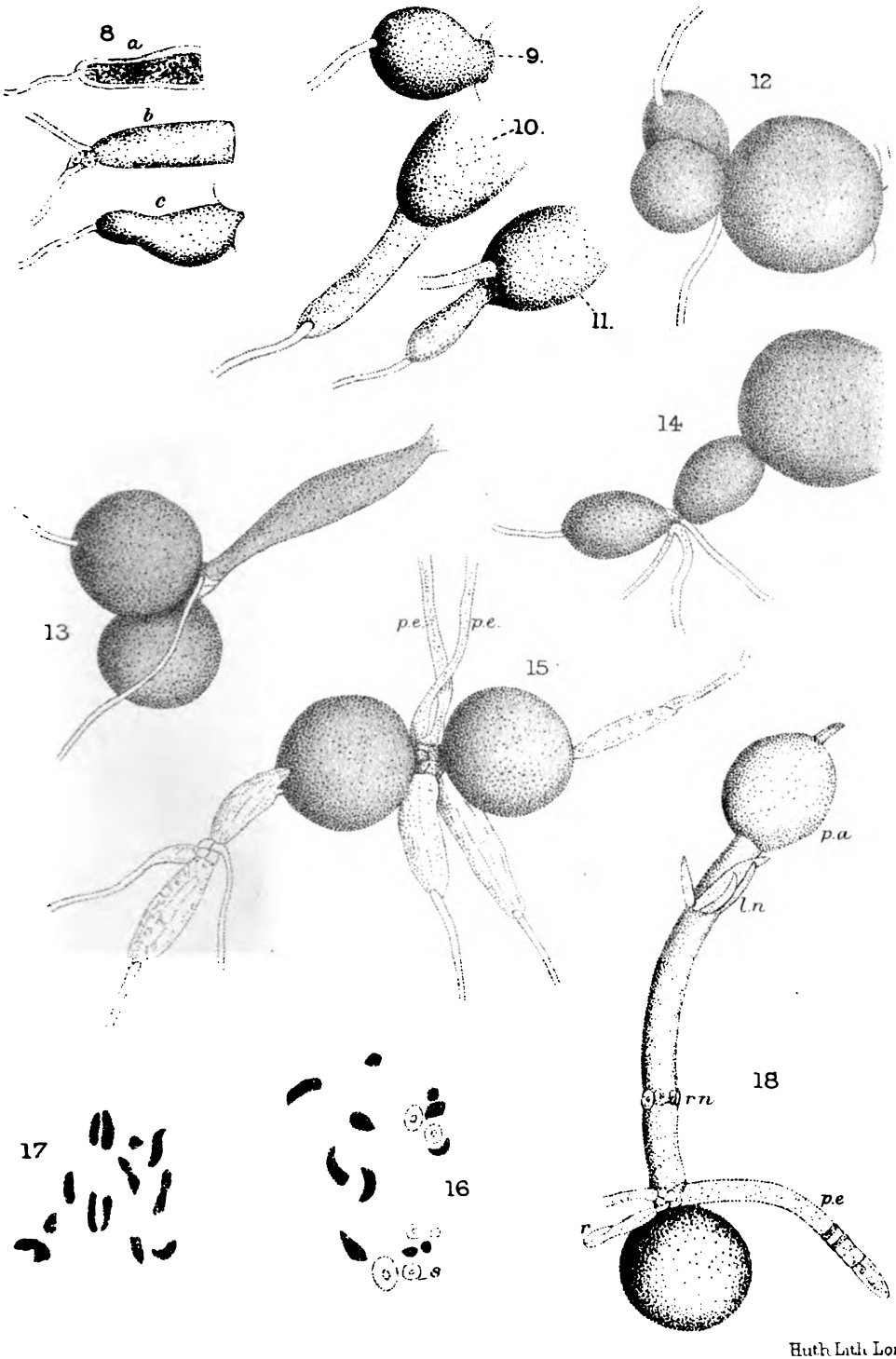

Huth Lith. London

Two new Triuridaceae, with some Remarks on the Genus Sciaphila, Blume.

BY

W. BOTTING HEMSLEY, F.R.S., F.L.S.

Keeper of the Herbarium and Library, Royal Botanic Gardens, Kew.

With Plates IX and X.

BLUME, in 1825, described a plant from Java under the name of *Sciaphila tenella*, the first member recorded of the singular group now known as the Triuridaceae. In 1851 he published an amplified description of the genus with incomplete descriptions of two proposed additional species. Since then botanists have described species, which they have referred to *Sciaphila*, from Ceylon, North-east India, Japan, the Malay Peninsula and Archipelago, New Guinea and New Caledonia, Brazil and Venezuela; making a total of about thirty species, most of which have been well figured. Studying these figures in connexion with the specimens of a plant of this natural order discovered by Mr. H. P. Thomasset in Mahé, Seychelles, I have been struck by the very great diversity in their floral structure, and I cannot myself accept the view that they all belong to one and the same genus. I do not propose making generic alterations from the figures alone, and I have not time to examine the whole of the materials; but I will discuss some of the various modifications in the structure or composition of the flowers, and compare the Seychelles plant with *Sciaphila tenella*.

All the members of the Triuridaceae are saprophytes or holosaprophytes, as Johow terms them, mostly having hairy roots, and they are very similar in aspect, being very slender, often almost capillary, and white, yellow, pink, coral-red, purple or violet in colour, with small scales in the place of leaves. They are mostly from 5 to 15 cm. high; but Spruce notes that *Sciaphila purpurea*, Benth., sometimes attains a height of 1·4 m. The usually unisexual flowers are small, occasionally very small; that is not more than ·5 mm. in diameter. The perianth is always regular, simple or uniseriate, with valvate segments nearly uniform in size and shape.

Taking *Sciaphila*, as limited or accepted by Bentham and Hooker,

[Annals of Botany, Vol. XXI. No. LXXXI. January, 1907.]

Beccari, Engler, Schumann and others, it presents a wide range of variation in floral structure, as here set forth.

Male flowers. Perianth 4- 6- or 8-lobed, rarely 3- or 5-lobed; the number of lobes not quite constant in the same species, nor in the same inflorescence, and sometimes differing in the two sexes. Perianth-lobes entire or rarely toothed at the tip (*S. caudata*, Pouls.), inappendiculate or with terminal clavate, comose, bearded or penicillate appendages. Staminodes none. Stamens 2, 3, 4 or 6, opposite the perianth-lobes; filaments none or very short, usually connate and central in the triandrous species; anthers 2- or 4-celled, dehiscing transversely (or longitudinally?); connective undeveloped or produced in a long, filiform tail above the anther-cells (*S. crinita*, Becc.). Pistillodes or rudimentary carpels none, or three or more, and either similar to the fertile ones or filiform (*S. andaiensis*, Becc.). The external appearance of the male flowers of the species just named and those of *S. crinita* is very similar, but the organs described by Beccari as rudimentary pistils are shorter and less finely pointed than the prolongations of the connective in *S. crinita*. The author does not, however, show them separately, as he does the stamens of *S. crinita*, and Schumann, without discussing the question, cites both species as having caudate stamens. 'Nuperrime autem cl. Beccari species duas *Sciaphilae*, nempe *S. crinitam* et *S. andaiensem*, descripsit, quarum connectivum non solum bene evolutum sed etiam in caudam filiformem apicem staminum longe superantem abiit, quae ante anthesin circa mediam antheram voluta adspectum peculiarem praebet.' I have not been able to check this, but I think Schumann probably judged by the figures only. However, Beccari himself was evidently in doubt as to the nature of these bodies in *S. andaiensis*, although his Latin description runs: '♂ pistilli rudimento in filamentis 3 linearibus e basi tripartito.' On the other hand in the Italian description which follows, he says: 'Questo corpo ha l'apparenza di un rudimento di pistillo; ma forse deve, come nella *S. crinita*, considerarsi come una produzione del connetivo delle antere.' In his key to the species, the two in question are placed under: 'Floris ♂ pistillodia 3 vel 1 tripartitum.' Of course there is the alternative of fusion of pistillodes and stamens[1].

Female flowers. Perianth presenting much the same modifications as in the male flowers, and either similar to or rarely different from that of the male in the same species. Staminodes none, or 3 or 6. Carpels always numerous, usually 30 or more, free, 1-celled, 1-seeded, dry or fleshy, dehiscent or indehiscent, smooth, verrucose, muricate or papillose; style

[1] Sir Joseph Hooker describes the male flowers of *S. khasiana*, as having three subulate pistillodes, but his original drawings, made from the living plant, show these bodies attached to the anthers, as though they were prolongations of the connective. There are no specimens of this plant at Kew, the published description having been made from the drawings.

ventral and basal, medial or subterminal, shorter or longer than the ovary, sometimes much shorter, sometimes much longer; stigma filiform, clavate or globose, naked, papillose or plumose.

Hermaphrodite flowers. In several of the species examined flowers have been found containing both stamens and pistils, and although the anthers sometimes contain pollen-grains and the ovaries ovules, it is uncertain whether both are ever perfect in the same flower. Some flowers may be functionally male; others functionally female. These quasi-hermaphrodite flowers are often irregular as to the number and disposition of the parts.

I think it will be generally conceded that the foregoing review of the range of modifications in the floral structure of plants referred to *Sciaphila* suggests further segregation. Bentham and Hooker, and also Engler, admit of only two genera in the order Triuridaceae, although Miers and others had long previously established five. Schumann more recently restored *Peltophyllum*, Gardn., and *Soridium*, Miers, South American forms.

A comparison of the Seychelles plant with Blume's description and figures of *Sciaphila tenella*, comes out as follows:—

	Sciaphila.	*Seychellaria.*
Male flowers.		
Perianth-lobes	bearded at the tips	naked
Staminodes	none	three
Stamens	six	three
Filaments	undeveloped	distinct
Anthers	two-lobed, dehiscing longitudinally	four-lobed, dehiscing transversely
Pistillodes	numerous	none
Female flowers.		
Perianth-lobes	bearded at the tips	naked
Staminodes	six	none
Carpels	fleshy	dry
Styles	shorter than the ovary	longer than the ovary

I have some doubt about the correctness of Blume's description and figures of the stamens. His description runs: 'Antherae sessiles, subrotundae, carnosae, paulum papillosae, loculis disiunctis ad margines sulco longitudinali dehiscentibus.' If this is an accurate description, *Sciaphila tenella* differs, in this respect, from all the other members of the order that I have examined, and all the figures that I have seen; but Beccari, although he does not discuss the point, evidently did not accept it as correct, because he refers several Bornean specimens having the characteristic anthers with transverse dehiscence to this species[1].

[1] Since the foregoing was written I have been able, through the courtesy of Dr. J. P. Lotsy,

Apart from this, however, the differences are sufficient, in my opinion, to justify the generic separation of the Seychelles plant from *Sciaphila*. As I have already stated, I shall not attempt to classify the thirty species, or thereabout, referred by various authors to *Sciaphila*, though, judging from the figures, I do not think it would be a difficult task.

Seychellaria, Triuridacearum novum genus ex affinitate *Sciaphilae*, Blume, a qua perianthii segmentis apice haud barbatis, florum ♂ staminodiis 3, staminibus 3, pistillodiis nullis, florum ♀ staminodiis nullis et stylo quam ovario longiore differt.

S. Thomassetii (species unica).

Planta saprophytica, carnosa, tenella, diaphana, concolor, pallida, praeter radices pilosas glabra, erecta, stricta, rhizomate repente squamoso ramoso, ramis ex squamarum parvarum axillis egredientibus subhorizontalibus pilosulis. *Caules* filiformes, pauciramosi, 10–12 cm. alti, vix 1 mm. crassi, squamis paucis ovato-lanceolatis acutis 2–3 mm. longis instructi. *Flores* carnosi, racemosi, racemis 6–12-floris, breviter pedicellati, 1–2 mm. diametro, unisexuales, monoici, inferiores feminei, paullo majores, superiores masculi, vel interdum nonnulli subbisexuales. *Perianthium* utriusque sexus saepius 6-partitum, rarius 4- vel 5-partitum; segmenta ovato-lanceolata, obtusa, alterna paullo minora, induplicato-valvata, inappendiculata vel apice obscure papillosa, demum arcte recurva. *Flores* ♂ triandri; staminodia 3, teretia, capitata, staminibus externa, longiora et iis alterna, perianthii segmentis alternis opposita; stamina centralia, perianthii segmentis alternis opposita, filamentis brevibus basi approximatis vel breviter connatis; antherae distincte 4-lobae, rima transversali dehiscentes, connectivo supra loculos haud producto. *Flores* ♀ sine staminodiis; carpella libera, numerosa (30–35), vix carnosa, rugulosa, in receptaculo conico sessilia, unilocularia, uniovulata; stylus ventralis, papillosus, ovarium multo superans. *Ovulum* erectum, anatropum, raphe ventrali. *Fructus* maturus non-visus.

SEYCHELLES: found growing at the base of trees in mountain forest at 600 m., Mare aux Cochons, Mahé, *H. P. Thomasset*, February, 1906. Herb. Kew.

It has already been mentioned that the quasi-hermaphrodite flowers of some of the Triuridaceae are occasionally irregular in structure in relation to the number and position of the parts. Some of the male flowers of *Seychellaria* are abnormal in having an extra, perfect or imperfect stamen or two; sometimes there are two or three stamens on contiguous instead of alternate segments of the perianth, and occasionally there are two or three imperfect carpels in the centre, and the staminodes are wanting. In one flower we found excrescences near the middle of perianth-segments, five of which were sessile and one stalked as shown in Figure 11 on Plate IX. It

Director of the National Herbarium, Leiden, to examine the type of *Sciaphila tenella*, Blume, with the result that I find his description accurate excepting that part relating to the anthers. The stamens of this species are different from those of any other member of the order that I have examined, but whether those seen are normal it is difficult to decide as the whole male flower is only about a millimetre in diameter. Miss M. Smith has drawn what we saw and it is described on p. 75.—W. B. H.

would appear that these small flowers are peculiarly subject to disturbances in their development.

In connexion with the Seychelles plant I have looked through the Triuridaceae at the British Museum, as well as those at Kew, and among the former is a specimen collected in the island of Aneiteum, New Hebrides, by John MacGillivray, in 1853 (or 1854, as labelled, but there is no evidence that MacGillivray visited the island a second time), which is apparently undescribed. With the permission of Dr. A. B. Rendle, the Keeper of the Botanical Department, I have been able to examine this plant and describe it, and excepting in the stamens, and perhaps the absence of staminodes from the female flowers, the floral structure is the same as that of *Sciaphila tenella*, Blume. I therefore place it provisionally in that genus.

Sciaphila aneitensis, *Hemsl.* species nova a *S. tenella*, Bl., floribus masculis triandris et carpellis numerosioribus haud verruculosis recedit.

Planta saprophytica, gracilis. *Caules* erecti, simplices, circiter 25 cm. alti, squamis paucis minutis instructi. *Flores* numerosi, racemosi, pedicellis brevibus recurvis, unisexuales, superiores masculi. *Perianthium* utriusque sexus 6-partitum, segmentis ovatis apice comoso-barbatis inflexis nunquam reflexis. *Flores masculi* circiter 3 mm. diametro, triandri; stamina perianthii lobis alternis opposita; filamenta brevissima; antherae bilobae, rima transversa dehiscentes; pistillodia numerosa, globosa. *Flores feminei* carpellis fere maturis circiter 5 mm. diametro; staminodia nulla? sed flores iuniores non visi; carpella 30–40, ovoidea, circiter 1 mm. diametro, laevia, quam stylus infra medium ventralis tertia parte longiora, stigmate minuto globoso papilloso.

New Hebrides: Aneiteum, *MacGillivray*, 1853. Herb. Mus. Brit.

Sciaphila tenella, Blume.

Figures 11 to 17, Plate X, represent two out of four of the plants which Blume apparently had under observation when he wrote his original description, together with their floral structure. All four plants appeared to be the same, though Miss Smith and I did not dissect flowers of all of them, and there is only one label on the sheet, which gives the locality, as near as we could decipher it, as van de Gunnung [i. e. Mount] Mendare. It is possible, however, that Blume had other specimens before him, because he gives more than one locality: 'Crescit in umbrosis Montis Menarae ac sylvarum Insulae Nusae Kambangae.'

The empty anthers of the three or four male flowers examined are 3-lobed, which is probably an abnormal condition, the normal condition being 4-lobed in many of those figured by Beccari and others, and in some cases the dehisced anthers look as though they were permanently 4-celled, as described by Miers in some other members of the order. And the anthers of *S. tenella* have the appearance of being permanently

3-celled. Be that as it may, there is little doubt that there is an error of observation in Blume's work, and that dehiscence by longitudinal slits does not occur in *Sciaphila*; and his 'fleshy anthers' were probably rudimentary carpels. Such a misconception of facts is not at all improbable where the parts are so extremely small.

BIBLIOGRAPHY.

BECCARI, O.: Le Triuridaceae della Malesia. Malesia, vol. iii, 1889, pp. 318–44, tt. 39–42.

BENTHAM, G.: On the South American Triurideae from the collections of Mr. Spruce. In Hooker's Kew Journal of Botany, vol. vii, 1855, pp. 8–12.

BENTHAM, G., AND HOOKER, J. D.: Triurideae. Genera Plantarum, vol. iii, 1883, p. 1001.

BLUME, C. L.: *Sciaphila tenella.* Bijdragen tot de Flora van Nederlandsch Indië, 1825, p. 514.

————: Triuridaceae. Museum Botanicum Lugduno-Batavum, vol. i, 1851, pp. 321 and 322, t. 48.

CHAMPION, J. G.: On two new Ceylon Plants related to *Sciaphila*, Blume. (*Hyalisma Ianthina* and *Aphylleia erubescens.*) In Calcutta Journal of Natural History, vol. vii, 1847, pp. 463–9, t. 5.

ENGLER, A.: Triuridaceae. Engler and Prantl, Die natürlichen Pflanzenfamilien, vol. ii, 1, 1889, pp. 235–8, mit 29 Einzelbildern.

HOOKER, J. D.: Flora of British India, Triurideae, vol. vi, 1893, p. 558.

————: Triurideae. Trimen's Handbook of the Flora of Ceylon, vol. iv, 1898, pp. 367–9.

JOHOW, F.: Die chlorophyllfreien Humuspflanzen. Pringsheim's Jahrbücher, vol. xx, 1889, pp. 475–525, tt. 19–22.

MACGILLIVRAY, J.: Hooker's Kew Journal of Botany, vol. vi, 1854, pp. 353–63.

MAKINO, T.: *Sciaphila japonica.* Botanical Magazine of Tokyo, vol. xvi, 1902, p. 211.

MIERS, J.: On the Family of Triuriaceae. In Transactions of the Linnean Society, vol. xxi, 1852, pp. 43–59, tt. 6 and 7.

POULSEN, V. A.: Bidrag til Triuridaceernes Naturhistorie. In Videnskabelige Meddelelser fra den Naturhistoriske Forening i Kjöbenhavn for Aarene 1884–6, pp. 161–79, tt. 12–14.

SCHUMANN, K.: Triuridaceae. In Martius's Flora Brasiliensis, vol. iii, pars 3, 1894, pp. 648–68, tt. 116 and 117.

SCHUMANN, K., and SCHLECHTER, R. In Schumann and Lauterbach's Nachträge zur Flora der deutschen Schutzgebiete in der Südsee, 1905, pp. 54–6, t. 2.

SCHLECHTER, R.: *Sciaphila neocaledonica.* Engler's Jahrbücher, vol. xxxix, 1906, p. 19.

EXPLANATION OF THE FIGURES IN PLATES IX AND X.

Illustrating Mr. Hemsley's paper on two new Triuridaceae.

PLATE IX.

Fig. 1. An old plant of *Seychellaria Thomassetii.* Natural size.
Fig. 2. Lower part of the same.
Fig. 3. A plant of *Seychellaria Thomassetii* in flower. Natural size.
Fig. 4. Lower part of the same.
Fig. 5. Portion of inflorescence.
Fig. 6. A flower-bud.
Fig. 7. A bract.

Fig. 8. A young male flower forcibly opened.
Fig. 9. A fully expanded male flower.
Fig. 10. A male flower opened out to show the attachment of the staminodes and stamens.
Fig. 11. A male flower with excrescences on the perianth-segments.
Fig. 12. A female flower with a tetramerous perianth.
Fig. 13. A nearly ripe fruit.
Fig. 14. A carpel.
Fig. 15. Section of the same showing the attachment of the young seed.
All the Figures except 1 and 3 much enlarged and some of them are more or less diagrammatic.

PLATE X.

Fig. 1. A plant of *Sciaphila aneitensis* shown in two sections. Natural size.
Fig. 2. A flower-bud.
Fig. 3. A male flower.
Fig. 4. A stamen.
Fig. 5. A young fruit showing the persistent perianth.
Fig. 6. The same seen from the side.
Fig. 7. A perianth-segment.
Fig. 8. Stigma.
Fig. 9. A carpel.
Fig. 10. A young seed.
Fig. 11. Plants of *Sciaphila tenella*. Natural size.
Fig. 12. An old male flower.
Fig. 13. Androecium and pistillodes.
Fig. 14. An old stamen.
Fig. 15. A female flower.
Fig. 16. A young fruit.
Fig. 17. A carpel.
All the Figures except 1 and 11 much enlarged and some of them are more or less diagrammatic.

HEMSLEY- ON TRIURIDACEÆ

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HEMSLEY—ON TRIURIDACEÆ

On the Existence of a Semi-permeable Membrane enclosing the Seeds of some of the Gramineae.

BY

ADRIAN J. BROWN.

DURING a recent investigation of the conditions governing the absorption of water by the ripe grain of certain cereals, some experiments with the grain of *Hordeum vulgare*, var. *caerulescens*, indicated that the embryo and endosperm of the grain are contained within a semi-permeable covering.

The grain of this variety of *H. vulgare* is characterized by a greenish-blue colour, due to the presence of a blue pigment in its aleurone cells. As the colour of this pigment, like litmus, is changed to red by acid, the grain is therefore naturally furnished with an indicator by means of which the access of free acid to its aleurone cells may be detected.

During some experiments with the blue variety of *H. vulgare*, in which the grain was steeped in a dilute solution (1 per cent.) of sulphuric acid, it was noticed that when the skins of the grain were punctured or otherwise damaged the colour of the aleurone cells of the grain adjacent to the damaged part of the skin rapidly changed to red, and that this colour transformation gradually spread to all the cells of the aleurone layer, demonstrating diffusion of acid throughout the endosperm of the grain. When, however, undamaged grain of the same variety of barley was steeped in a similar solution of acid it was observed that although the contents of the grain became soft and swollen from absorption of water, the aleurone cells retained their original blue colour, indicating that the acid had not obtained access to the interior of the grain. It was further noticed that this condition remained for a week or even longer, if germination of the grain was prevented or delayed by keeping it submerged in the acid steeping liquid. If, however, germination took place, with consequent rupture by the growing roots of the skins of the grain where they protect the base of the embryo, the aleurone cells of the grain speedily changed to a red colour, indicating that acid had diffused through the tissues of the embryo into the endosperm of the seed.

[Annals of Botany, Vol. XXI. No. LXXXI. January, 1907.]

These observations regarding the behaviour of the grain of the blue variety of *H. vulgare* when steeped in dilute sulphuric acid therefore indicated that water alone obtains entrance to the grain under these conditions if its coverings remain intact, and that consequently the coverings of the grain behave as a semi-permeable or selective membrane with regard to an aqueous solution of sulphuric acid. But as this conclusion was based solely on indications furnished by the colour reaction with acid of the blue pigment of the aleurone cells of the grain, it was evidently desirable to test it by other methods of investigation.

In further experiments grains of the blue variety of *H. vulgare* were steeped in a 1 per cent. solution of sulphuric acid for periods of time varying from 24 to 72 hours, and subsequently were cut—some longitudinally, and others transversely—into sections. On testing the reaction of these sections with a solution of methyl-orange no indication of the presence of free acid was obtained in any portion of the seed within the testa—the coverings of the grain, however, exhibited an acid reaction. In all the experiments the presence of water was demonstrated within the seed coverings, both by the swollen and soft condition of the endosperm and also by the turgid condition of the cells of the embryo.

It was evident, however, that more conclusive proof that the coverings of the grain functioned as a semi-permeable envelope would be obtained, if, following steeping the grain in a solution of sulphuric acid, concentration of the solution of acid was demonstrated—for manifestly concentration must take place if water only is absorbed by the grain under these conditions.

Experiments with barley grains, in which they were steeped in normal (4·9 per cent.) sulphuric acid, and the remaining acid solution was subsequently titrated with standard alkali, readily demonstrated that a marked concentration of acid in the solution takes place under these conditions. But it appeared more satisfactory to establish, if possible, that the concentration which takes place is proportional to the volume of water abstracted from the solution of acid by the barley grains during steeping.

The following experiment was made with this object in view. 10·899 grms. of air-dried barley grains were in the first instance steeped in 15 c.c. normal (4·9 per cent.) sulphuric acid for 3 hours, and afterwards the grains were drained, well dried in filter-paper, and reweighed. The weight obtained was 12·509 grms. This preliminary treatment of the grain was carried on in the first instance to neutralize a feeble basic condition apparently natural to the skins of the grain, and secondly, in order to correct as nearly as possible for the weight of moisture adhering to the skins of the grain after draining and drying in filter-paper.

Following this preliminary treatment the grain was again steeped in 15 c.c. normal sulphuric acid and allowed to remain for 48 hours.

Subsequently after draining and drying the grain in filter-paper as before it was found to weigh 14·459 grms. The grain had therefore absorbed 1·950 grms. of moisture from the normal acid in which it had been steeped. This weight would represent 1·950 c.c. water abstracted from the original volume of normal acid if the skins of all the grains of barley employed were undamaged and had permitted the passage of water only through them; but on examination it was found that the skins of 7·2 per cent. of the grains employed had been injured and had admitted the passage of both acid and water to the interior of the grains. Consequently the volume of pure water abstracted from the acid solution and tending to concentrate it is 1·81 c.c. (1·95 c.c.–0·14 c.c.). On subtracting this amount from the original volume of 15 c.c. of normal acid employed, 13·19 c.c. remain as the diminished volume of the acid solution calculated from the weight of water absorbed by the grain.

On directly titrating 10 c.c. of the acid solution with normal (4·0 per cent.) caustic soda, it was found that 11·1 c.c. were required for neutralization; hence 11·1 c.c. of the original normal acid have been concentrated to a volume of 10 c.c., which is equivalent to a concentration of the original 15 c.c. employed to 13·51 c.c.

Therefore, from the weight of water absorbed by the grain, a concentration of the original 15 c.c. of normal acid to 13·19 c.c. has been found, and by direct titration a concentration of 13·51 c.c.—close agreement considering the relatively large errors present in the method of experiment which it was found necessary to adopt.

In order to demonstrate still more conclusively that a solution of sulphuric acid is concentrated by the semi-permeable property of barley grains, four separate quantities of grain were steeped successively for 24 hours each in the same solution of normal sulphuric acid in order to intensify the concentration effect. On subsequently titrating the acid solution which remained unabsorbed by the grain its strength was found to have increased from the original concentration of 4·9 per cent. to 7·62 per cent. H_2SO_4.

The experiments described demonstrate conclusively that a separation of water and sulphuric acid is effected when undamaged grains of barley are steeped in solutions of sulphuric acid not exceeding a concentration of 4·9 per cent. As it appeared desirable, however, to ascertain if a similar phenomenon is exhibited when barley grains are steeped in more concentrated acid, some grains of the blue variety of *H. vulgare* were immersed in solutions of 9·0, 18·0, and 36·0 per cent. H_2SO_4 for 44 hours.

On subsequent examination it was found in all the experiments that the original blue colour of the undamaged grains remained unchanged. Further examination by means of methyl-orange also demonstrated that no free acid had penetrated to the interior of the grains. When the grains

were cut, however, a difference was observed in the conditions of their contents in respect of the amount of water absorbed during steeping in the solutions of acid. The endosperms of the grains steeped in 9 per cent. sulphuric acid were soft, and the tissues of the embryos were turgid through absorption of water. The endosperms of the grains steeped in 18 per cent. acid were not so soft, and the embryos, although distinctly moist, were much less so than in the experiment with weaker acid. In the case of the grains steeped in 36 per cent. acid, both their endosperms and embryos appeared to be as hard and dry as they were previous to steeping in the acid.

It is evident when a semi-permeable membrane capable of separating water and sulphuric acid is in action some such results as those described are to be expected, and they suggest some interesting questions in connexion with osmosis which are reserved for future investigation.

In order to obtain additional proof that solutions of sulphuric acid of such high concentration as 18 and 36 per cent., employed in the experiments just described, had not gained access to the embryos of the barley grains and destroyed their vitality, some of the grains which had been steeped in these two solutions of acid were well washed in water and subsequently placed under conditions favourable for germination. In a few days all were in vigorous growth.

Following on the experiments with sulphuric acid which have been described, some experiments were made in which barley grains were steeped in solutions of various salts in order to ascertain if water alone passes into the interior of the grains under these conditions.

5 per cent. solutions of cupric sulphate, ferrous sulphate, potassium chromate, silver nitrate, and potassium ferrocyanide, were employed with the result that no trace of any of these salts was found within the coverings of the grains after three days' steeping, although in all cases the embryos and endosperms of the grains were softened and distended with water.

Experiments were also made with solutions of sodium hydrate of varying concentration. Solutions of 1 per cent. strength and upwards destroyed the coverings of the grain and ultimately disintegrated the grain completely.

The coverings of the grain were, however, found to withstand the action of a 0·5 per cent. solution, and under these conditions, although water passed from the solution into the grain, sodium hydrate was excluded.

Experiments with solutions of hydrochloric acid of normal (3·65 per cent.) strength also showed that this acid, like sulphuric acid, is excluded from the interior of the grains.

With nitric acid, however, somewhat different results were obtained. When barley grains were steeped in a 1 per cent. solution of this acid it was found that the acid was excluded during 24 hours' immersion, and in

the case of a small number of the grains a similar result was obtained after 48 hours' steeping, but eventually it was found that all the grains admitted acid [1].

A similar condition was noticed, but after a much shorter interval of time, when the grains were immersed in 5 per cent. nitric acid. From the appearance of the grains after steeping, there is reason, however, to believe that the passage of nitric acid to the interior of the grains does not result from a selective action of their semi-permeable envelope, but from destruction of the semi-permeable property of the envelope by the chemical action of the acid.

Apparently, however, an instance of the envelope exhibiting a power of selection for matter other than water is found in the passage of iodine into the barley grain.

When barley grains are steeped in a 1 per cent. solution of iodine in iodide of potassium (5 per cent.) solution for 24 hours, the passage of iodine through the envelope into the interior of the grains is evidenced by the contents of the cells of the embryo and of the aleurone cells being stained brown, and also more markedly by the starch granules of the starch-containing cells of the endosperm being stained blue.

The latter phenomenon, which renders it easy to study the manner in which iodine passes into the grain, indicates that it penetrates all parts of the skins enveloping the endosperm at approximately the same velocity, with the exception of the part in the neighbourhood of the ventral-furrow, through which it appears to pass with difficulty.

In the first instance it seemed probable that access of iodine to the interior of the grain was only obtained after destruction of the semi-permeable property of the envelope by the chemical action of the iodine, but experiments in which grains of barley previously stained with iodine were steeped in a solution of sodium hyposulphite, appeared to show that such is not the case. Under such condition, if the semi-permeable character of the envelope of the grains was destroyed, the sodium hyposulphite would diffuse into the grains and discolour the starch-containing cells of their endosperms which had previously been coloured with iodine; and, in confirmation of this, direct experiment showed that when grains of barley stained with iodine were steeped in a solution of sodium hyposulphite after their skins were punctured or otherwise damaged, the sodium hyposulphite diffused into the grains and discoloured them. On the other hand, when iodine-stained grains with uninjured skins were steeped in a solution of sodium hyposulphite, no discoloration was observed even after the grains had been immersed in the solution for five days.

[1] It appeared that entrance of the acid was always obtained at the germ, or proximal, end of the seed.

The experiments described above demonstrate that the embryo and endosperm of the barley grain are enclosed within an envelope through which water and iodine can pass to the interior of the grain, but through which certain acids and salts cannot pass. As there appeared to be no known instance of the occurrence in the vegetable kingdom of a membrane other than one of living protoplasm, which possessed a marked semi-permeable property, it seemed very desirable to inquire if the semi-permeable property of the envelope of the barley grain was a function of living protoplasm, although from the first it appeared very improbable that it was so when the conditions ruling in some of the experiments already described were considered.

In order to demonstrate the semi-permeable character of living protoplasm by plasmolysis, solutions either of inert salts, or of such bodies as cane sugar, which do not exert an injurious action on the protoplasm of the living cell experimented with, must be employed, for otherwise, as the solute must come into direct contact with the protoplasm of the cell in order to exert its osmotic influence, its vitality would be destroyed and it would cease to function as a semi-permeable membrane. But in some of the previous experiments it has been shown that the semi-permeable property of the envelope of the barley grain is exhibited with solutions of sulphuric acid of very high concentration, and it appears inconceivable that such strong acid can come in contact with living protoplasm without destroying its vitality at once. Moreover it has been shown that when solutions of such poisons for protoplasm as silver nitrate and cupric sulphate are employed these salts are excluded from the interior of the barley grain. Additional evidence that living protoplasm does not act as the semi-permeable membrane of the barley grain is also furnished by the results of the experiments with iodine, which have already been described. In these experiments it was shown that iodine penetrates the coverings of the grain, and yet on subsequent immersion of the grain in a solution of sodium hyposulphite the coverings through which iodine has already passed prevent the passage of the salt. To regard the selective character of the coverings as depending on the action of living protoplasm after the passage of a strong poison like iodine through them seems unreasonable.

Conclusive proof that the semi-permeable character of the envelope surrounding the grain does not depend on the activity of living protoplasm was, however, obtained by experiments conducted with grains of barley after they had been subjected to the action of boiling water. Some barley grains were immersed in boiling water, and at intervals of 5, 10, 30, 60, and 120 minutes a number of the grains were removed. On examination it was found that the skins of all the grains which had been steeped in boiling water for 120 minutes were ruptured and their contents were extruding, and of those grains similarly treated for 60 minutes all but one

were ruptured. In the case of the grains boiled for 30, 10, and 5 minutes all remained whole.

Following on the treatment with boiling water the grains from the three last experiments, the skins of which were apparently uninjured, were steeped in normal sulphuric acid for 48 hours. On subsequent examination it was found that no trace of acid had penetrated to the interior of the grains except in a very few instances in which the skins of the grains had been accidentally damaged. In order to obtain additional evidence that life in the grains had been destroyed by the treatment to which they had been subjected, some of the grains which had been immersed in boiling water for the shortest period of time (5 minutes) were placed under conditions favourable to germination, with the result that they exhibited no signs of life.

The above experiments therefore show conclusively that the semi-permeable property of the envelope of the barley grain is not a function of living protoplasm.

A study of the nature and position of the semi-permeable envelope enclosing the barley grain was then commenced. Previous observations regarding the behaviour of the blue pigment in the aleurone cells of grains of *H. vulgare*, var. *caerulescens*, when steeped in an acid solution, demonstrate that the semi-permeable envelope must occupy a position which is external to the aleurone cells of the endosperm, and also that it must enclose both the embryo and endosperm of the grain.

The envelope enclosing the embryo and endosperm of the barley grain apparently consists[1]: (1) of the pales, originally the floral envelope; (2) of the pericarp, composed of several layers derived from the component parts of the walls of the ovary; and (3) of the spermoderm, composed of the ovular integuments together with the outer layer of the nucellar tissue. The semi-permeable property of the grain therefore should be located in one or more of these coverings.

The pales of the grain do not function as the semi-permeable covering, for after their removal the grain exhibits its semi-permeable property equally as well as before. The property also does not appear to be a function of any part of the pericarp, for the layers of cells composing this covering are disintegrated when the barley grain is digested in a 36 per cent. solution of sulphuric acid without the semi-permeability of the grain being destroyed. (See above, p. 81.)

The spermoderm of the grain, however, resists the action of 36 per cent. sulphuric acid in a very remarkable manner, and hence this covering or one of its component layers probably constitutes the semi-permeable membrane of the barley grain.

[1] See 'Développement et constitution de l'endosperme de l'orge'. W. Johannsen. Comptes Rendus des travaux du Laboratoire de Carlsberg. Vol. 2, 1884, 63.

In order to investigate this point further, grains of *H. vulgare*, var. *caerulescens*, and of several varieties of *H. distichum* were steeped for 48 hours in solutions of methyl-violet and of fuchsin, and also in solution of various salts which could be readily traced by micro-chemical means, and subsequently sections of the grains were prepared for the purpose of studying microscopically the limit of passage of the reagents employed through the coverings of the grains.

The best results were obtained with grains which were in the first instance steeped in a 3 per cent. solution of silver nitrate for 48 hours, and afterwards steeped for 48 hours in a 5 per cent. solution of sodium chloride. By this means the limit of penetration of the silver nitrate through the skins of the grain was rendered visible by its precipitation as silver chloride in the cellular tissues and subsequent blackening on exposure to light.

Microscopic examination of sections of barley grains treated in this manner demonstrates that silver nitrate passes through the pales and pericarp into the spermoderm, which, in part at least, is coloured by the silver salt. A thin layer of uncoloured membrane, however, remains between the stained portion of the spermoderm and the walls of the aleurone cells of the endosperm. Probably this layer of the spermoderm is derived from the epidermis of the nucellus, but its identity has not been satisfactorily determined at present.

When transverse sections of a barley grain treated with silver nitrate and sodium chloride are examined in the neighbourhood of the funicle a very well-defined line is found, indicating that the silver salt, after penetrating the spermoderm some distance, stops abruptly at the outermost layer of the sheaf-like mass of empty cells, which are usually regarded as the remains of the cells of the nucellus; but it appears undesirable to draw any definite conclusion from this observation owing to the complex character of the envelope of the barley grain in the neighbourhood of the funicle.

There seems to be no doubt that the semi-permeable property of the barley grain centres in the spermoderm, but whether the property is confined to one of its layers only has not been definitely determined as yet.

As it appeared interesting to ascertain if the grain of other of the Gramineae than *Hordeum* exhibited the phenomenon of semi-permeability, grains of *Avena*, *Triticum*, and *Secale* were steeped in a normal solution (4·9 per cent.) of sulphuric acid for 48 hours, and subsequently examined. It was found that they all evidenced the possession of a semi-permeable covering, as in the case of *Hordeum*. In the case of *Triticum* and *Secale*, however, a much larger proportion of damaged grains not capable of excluding acid were found as compared with *Hordeum* and *Avena*.

Apparently this is due to the grain of *Triticum* and *Secale* not retaining the pales as a protective covering, as in the case of *Hordeum* and *Avena*, and consequently to the skins which compose the pericarp and spermoderm of the grain of *Triticum* and *Secale* being more exposed to accidental injury.

Conclusion.

This paper gives an account of the preliminary work of an investigation which is being continued, and although the results so far obtained suggest further discussion both from a botanical and a physico-chemical point of view, it appears desirable at present to await the results of experimental work which is proceeding. The following summary appears, however, to be justified :—

1. The grain of *Hordeum* is enclosed within a semi-permeable or selective covering, which permits the passage of water and iodine to the interior of the grain, but which prevents the passage of sulphuric and hydrochloric acids, and all salts of metals at present examined, when they are in aqueous solution.

2. The semi-permeable property of the covering of the grain of *Hordeum* is not due to the action of living protoplasm.

3. The semi-permeable property of the covering of the grain of *Hordeum* is located in the spermoderm of the grain.

4. The grain of *Avena*, *Triticum*, and *Secale* is enclosed in a semi-permeable covering apparently similar to that of the grain of *Hordeum*.

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The Structure of the Palaeozoic Seeds, Trigonocarpus Parkinsoni, Brongniart, and Trigonocarpus Oliveri, sp. nov.[1] Part I.

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With Plates XI–XIV.

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I. INTRODUCTION.

IN January last we published a preliminary note on the structure of *Trigonocarpon olivaeforme*[2], giving a short account of a reinvestigation of a large number of the seeds. The object of the present paper is to illus-

[1] [The investigation recorded in the present paper was carried out jointly by my colleague and myself at the Jodrell Laboratory. The part of the paper now published, giving the results of our joint observations, has been written entirely by Mr. Maslen. In the second part we hope to discuss the more general questions arising out of our work.

D. H. SCOTT.]

[2] Scott and Maslen, 1906.

trate and describe in detail the structures there referred to, to describe a new species of *Trigonocarpus*[1], to briefly describe the specimens which are preserved as structureless casts and impressions and compare them with those in which the internal structure is preserved, and to discuss the probable affinities of *Trigonocarpus* and the general bearing of our work.

Among the many forms of seeds which occur in the British Coal-Measures none are more familiar to geologists and palaeobotanists than the more or less triangular nut-like seeds referred to Brongniart's genus *Trigonocarpus*. This name (or *Trigonocarpum*) was originally applied by Brongniart, in his *Prodrome d'une Histoire des Végétaux fossiles*[2], to French specimens, on account of their characteristic three-ridged form as seen in the ordinary sandstone casts, and equally well in transverse sections of the petrified seeds preserved in the calcareous concretions of the Lower Coal-Measures.

In the most common mode of preservation, the familiar three-angled 'nuts' of the miners were long ago shown by Hooker and Binney[3] and by Williamson[4] to be nothing more than internal casts of the cavity of the seed, and to be often surrounded by a thin coaly layer which was correctly interpreted as the remains of the testa. These internal structureless casts are found in all parts of the Coal-Measures. Wild[5] instances the Peel Delf rock, near Worsley, in the Upper Coal-Measures, and the Bardsley Delf rock, near Ashton-under-Lyne, in the Middle Coal-Measures, as localities which have yielded a great many examples of these triangular internal casts, while in the Lower Coal-Measures *Trigonocarpus* is not uncommon in a petrified form. Some of the specimens exhibited at the Natural History and other Museums show hundreds of examples of *Trigonocarpus* on one slab of rock, and thus serve to illustrate in a striking manner the abundance of these seeds and of the plants on which they were borne in later Palaeozoic times.

Trigonocarpus occurs also at corresponding horizons in many parts of Europe. The French Stephanian (corresponding to our Upper Coal-Measures), especially at Grand'Croix, near St. Étienne, has yielded numerous types of Gymnosperm seeds, many of which were originally described by Brongniart[6]. These silicified Carboniferous seeds from St. Étienne have also been studied by Renault, and quite recently by Prof.

[1] Some reason should be given for our use of the form *Trigonocarpus* in this paper instead of *Trigonocarpon*, which was the name used in our preliminary note. The name of this seed has, unfortunately, been spelt differently by different authors. Brongniart himself in his original 'Prodrome' uses *Trigonocarpum*, in his 'Tableau' *Trigonocarpon* is used, while in the memoir on the St. Étienne seeds the genus appears as *Trigonocarpus*. Lindley and Hutton used *Trigonocarpum*, while Williamson adopted *Trigonocarpon*. Prof. F. W. Oliver, F.R.S., in his recent papers on some of the French seeds uses *Trigonocarpus* and for the sake of uniformity among present-day observers we have adopted the same form.

[2] Brongniart ('28). [3] Hooker and Binney ('55). [4] Williamson ('77), p. 251.

[5] Wild ('00), p. 435. [6] Brongniart ('74), ('81).

F. W. Oliver, who has commenced a re-examination of the seeds and from whom valuable papers have already appeared on *Stephanospermum* [1], *Trigonocarpus pusillus* [2], and other forms.

In Brongniart's memoir on these silicified seeds from Grand'Croix, published in 1874 [3], they are divided into two series, according to shape, as follows:—

(A) *Graines à symétrie binaire, plus ou moins aplaties et bicarénées.*

(B) *Graines à symétrie rayonnante autour de l'axe à trois, six, huit divisions ou à section circulaire.*

For these series Prof. Oliver has proposed the names Platyspermeae and Radiospermeae respectively [4].

The Radiospermeae are further divided into several groups based on the form of the transverse sectio·, circular, triangular, hexagonal, or octagonal; and the three-angled forms are referred to three genera, *Trigonocarpus*, *Tripterospermum*, and *Pachytesta*. Of these genera, *Trigonocarpus* and *Tripterospermum* appear to be so nearly alike as to be difficult of separation. Indeed, the first British specimens of *Trigonocarpus* of which the internal structure was described—by Hooker and Binney in 1855 [5]—were regarded by Brongniart himself as probably belonging to his genus *Tripterospermum*. The specimens afterwards described by Williamson under the name of *Trigonocarpon olivaeforme* [6] undoubtedly represent the same form as the Hooker and Binney seed, and in fact nearly all the structural specimens which we have examined from the British Coal-Measures, and they are numerous, are so generally similar in size, form, and structure as to lead us to the conclusion that they probably belonged to the same species of plant.

It is important, therefore, at the outset of our investigations, to critically consider the two genera *Trigonocarpus* and *Tripterospermum*, and to decide to which form our common British specimens should be referred.

The characters used by Brongniart for the separation of these two genera are given in the following extract. *Tripterospermum* is described as of the same general form as *Trigonocarpus*: 'Mais ce testa, très-épais, se prolonge en trois ailes très-saillantes, et est composé de deux couches très-distinctes: l'interne est formée d'un tissu serré, très-coloré et très-opaque, composé de cellules diversement dirigées; l'extérieure, plus large, est constituée par un tissu plus lâche et plus transparent' [7]. One of the distinguishing characters here given, viz. the double-layered testa, is now known to be shared by Brongniart's *Trigonocarpus pusillus*, as shown by Prof. Oliver in his recent re-examination of this seed from Grand'-Croix [8]. Prof. Oliver shows that the compact layer of the testa is

[1] Oliver ('04) (1).
[2] Oliver ('04) (2).
[3] Brongniart ('74).
[4] Oliver ('04) (1), p. 389.
[5] Hooker and Binney ('55).
[6] Williamson ('77).
[7] Brongniart ('74).
[8] Oliver ('04) (2), p. 98.

overlaid by two or three layers of thin-walled cells, which in all probability represent but a small portion of a much more extensive tissue, so that, according to this character, *Trigonocarpus pusillus* (Brongn.) is a *Tripterospermum*.

The other distinguishing feature of *Tripterospermum*, i. e. the more wing-like form of the ridges of the hard testa, remains to be considered. The difference would be best seen by a comparison of transverse sections of the body of the seed in the two genera. Comparing our common British form, described in this paper as *Trigonocarpus Parkinsoni* (see Pl. XI, Fig. 5), with the figures given by Brongniart, it seems to agree (as was evidently thought by this author himself) rather more closely with *Tripterospermum* than with *Trigonocarpus*.

Should we, then, describe our British forms as belonging to the genus *Tripterospermum*? We have already seen that the degree of prominence of the three principal ridges of the testa is the only remaining character by which to distinguish the two genera. Among our British forms there is some variation in this respect, not only in the principal ridges, but also in the less prominent ribs which occur between them, as will be more fully discussed later. Prof. Oliver, in his memoir on *Stephanospermum*, one of the radiospermic Gymnospermous seeds from St. Étienne, and in a comparison of this form with other related Palaeozoic seeds, after pointing out that the seed described by Williamson as *Trigonocarpon olivaeforme* unquestionably belongs to the same affinity as the Hooker and Binney specimens, says: 'Consequently, for the present discussion, both these *Trigonocarpons* may conveniently rest in the Brongniartian genus *Tripterospermum*'[1]. Somewhat later, in his *Notes on Trigonocarpus and Polylophospermum*, discussing the same subject, Prof. Oliver says that 'In any case the structural feature used by Brongniart to separate *Trigonocarpus* and *Tripterospermum* seems unimportant'[2].

We concur in this opinion, and shall therefore describe our forms as belonging to *Trigonocarpus*. We are the more disposed to do this as this name has been extensively used in this country by Williamson and others for petrified specimens and casts similar to those described in this paper, and so has become well known to every one interested in fossil plants. *Tripterospermum* has never been used for British forms.

We may now consider the question of the specific name which should be given to the common form. This question is made the more difficult of solution owing to the difficulty of accurately comparing the structural specimens preserved completely embedded in a matrix with the much more common form of preservation as a structureless cast. The specific names have usually been first applied to the casts and afterwards to structural specimens which appeared to agree in shape and size.

[1] Oliver ('04) (1), p. 391.

[2] Oliver ('04) (2), p. 97 note.

According to Mr. Kidston[1] the earliest figured specimens of *Trigonocarpus* are those given in Parkinson's *Organic Remains*[2], published in 1811. These are the familiar internal nut-like casts to which Brongniart, in his *Prodrome*[3], gave the name *Trigonocarpum Parkinsoni.* This name appears to be the earliest applied to British specimens.

The other familiar specific name, *olivaeforme*, seems to have been first used by Lindley and Hutton for casts[4], and afterwards by Williamson[5] for specimens showing internal structure.

Lindley and Hutton figure and describe several species of *Trigonocarpus* (their *Trigonocarpum*) from the casts. Some of these forms differ from our common form in size and shape to such an extent that we feel no doubt as to their being distinct species, but two somewhat similar forms are figured as *T. Noeggerathi* and *T. olivaeforme.* Internal casts of *T. Noeggerathi* are said to be provided with six longitudinal ridges instead of three as in *T. olivaeforme.* Internal casts of any of our specimens would certainly have only three ridges, so that *T. Noeggerathi* may be excluded. Moreover, according to Mr. Kidston, *T. Noeggerathi* is an uncommon seed in Britain[6].

It rests, then, between *T. Parkinsoni* (Brongn.) and *T. olivaeforme* (L. and H.), which Mr. Kidson, after examination of the casts in the Natural History and other Museums has pronounced to be really indistinguishable from one another[7]. If this identification as one species is correct—and as far as our own observations go they are in support of it—*T. Parkinsoni* (Brongn.), as the earlier name, should be used. The common British specimens will therefore be described in this paper as *Trigonocarpus Parkinsoni.*

Lindley and Hutton also figure, under the name of *Carpolithes alata*[8], specimens which show not only the internal cast of the cavity of the body of the seed, but also that of the elongated micropylar canal, and an impression of the testa of the seed, as shown in Pl. XII, Figs. 16 and 17, and Pl. XIII, Fig. 18, of this paper. These specimens Mr. Kidston also identifies with *Trigonocarpus Parkinsoni*[9], and we think that there is no doubt that they do represent a more complete form of preservation of the same or a similar form to that represented by the ordinary internal casts and the petrified specimens.

Thus we now know the common *Trigonocarpus* in three conditions:—

(1) As the common internal casts of the central cavity of the seed, the actual tissues having disappeared completely, or being represented only by a layer of structureless coal.

[1] Kidston ('86), p. 216. [2] Parkinson ('11). [3] Brongniart ('28).
[4] Lindley and Hutton ('31–'37), vol. ii, Pl. 222, Figs. 1, 3.
[5] Williamson ('77). [6] Kidston ('86), p. 219. [7] Kidston ('86), pp. 218, 219.
[8] Lindley and Hutton ('31–'87), vol. ii, Pl. 87. [9] Kidston ('86).

(2) As structureless casts and impressions showing, in addition to the ordinary cast of the body of the seed, the long micropyle and testa.

(3) As completely petrified specimens in which the internal structure is more or less perfectly preserved.

II. Previous Work on the British forms of Trigonocarpus.

We propose next to pass in review the work which has previously been done on the British forms of *Trigonocarpus*, especially on the petrified specimens showing internal structure. The earlier papers have been referred to in the preceding section, but a connected, somewhat fuller account may be useful before passing to our own work. Lindley and Hutton, in the *Fossil Flora* [1], deal only with the ordinary structureless internal casts, and with impressions showing the outer coats, but without internal structure, which they described as *Carpolithes alata*. The internal casts are referred to several different species by differences of shape, number of longitudinal ridges and size. Some of these casts, *T. olivaeforme* and *T. Noeggerathi*, are common forms and closely resemble in size and shape the most commonly found petrified specimens as described in the present paper. These 'species' have already been discussed in this paper (p. 93). Other forms such as *T. ovatum* and *T. Dawesii* are less common as internal casts, and from their greater size and difference in shape almost certainly represent distinct species.

An important paper, and the earliest one dealing with the internal structure of the common British form of *Trigonocarpus*, is that by Hooker and Binney published in 1855 [2]. The specimens came from calcareous nodules in the lower part of the Lancashire Coal-field, and the slides are now preserved in the Geological Department of the Natural History Museum and in the Binney Collection at Cambridge. The authors give a good general account of the organization of the seed, as well as a short account of the relation of the petrified specimens to the common casts. The latter are described as being merely casts of the cavity of the seed, i.e. of the cavity within the hard integument. The two layers forming the coat of the seed are distinguished, and the microscopic structure of each described in some detail.

The rounded (basal) end of the seed is described as being surrounded by an annular ridge, an outgrowth of the harder, inner, layer of the testa, whilst at the other end of the seed the integuments are prolonged as a conical cylindrical or trigonous beak traversed by a narrow canal. The base of this micropylar beak is said to be surrounded, in some cases, by an annular ridge, but from our own observations of the original slides and many others of the seed, we are led to think that this appearance may

[1] Lindley and Hutton ('31–'37).

[2] Hooker and Binney ('55).

be explained as a result of obliquity of the plane of section, which will produce a deceptive appearance of an annular ridge surrounding the upper part of the seed, as will be more fully explained in a later part of this paper.

Some of the Hooker and Binney slides show the tracheal elements of the nucellus in a well-preserved condition, and these are described and figured in their paper. All the Hooker and Binney slides are longitudinal sections.

The next description of the structure of *Trigonocarpus* was by Williamson in 1877 [1], under the name of *Trigonocarpon olivaeforme*. He described and figured a considerable number of vertical and transverse sections of good specimens from the Oldham deposits, at about the same geological horizon as that from which Mr. Binney obtained his examples. The specimens were cut into series of many longitudinal and transverse slices which are figured in natural size. The most instructive of these is a series of transverse sections (see Williamson's paper, Figs. 100–8), which start from the lower portion of the micropylar tube, and traverse successively lower zones of the seed, and which were very useful for comparison with the longitudinal sections which had previously been figured by Hooker and Binney. With the aid of these transverse sections, Williamson gave the first clear explanation of the origin of the ordinary triangular internal casts. He also described in detail the microscopic structure of the inner hard layer of the seed-coat which he calls the endotesta (=our sclerotesta) and gives a good figure.

In 1900 Mr. George Wild published a paper: 'On New and Interesting Features in *Trigonocarpon olivaeforme* [2]' from observations based on a number of new slides which have since come into Dr. Scott's possession, and some of which have been made use of in the preparation of our paper. From a study of thirteen consecutive transverse sections, Wild was able to show (1) that the micropylar beak was very much longer than had previously been suspected, being nearly or quite equal in length to the body of the 'fruit' itself; (2) that the micropylar beak retains its characteristic triangular cavity nearly up to the apex, and (3) that the long micropyle was apparently winged, the soft (outer) layer of the testa being developed in a flattened form on both sides of the central harder tissue. This interesting feature will be discussed later on in this paper.

Wild also emphasized the fact of the frequent association of *Trigonocarpus* with *Myeloxylon*, which is now known to be the petiole of *Medullosa*. The block from which Wild's slides were cut abounds in petioles, roots and other parts belonging to *Medullosa*, and most of the other preparations which we have seen agree in this respect. In Wild's slides the limiting layers of the soft testa of the seed are often well preserved, and Wild pointed out the close similarity in structure between these and the outer

[1] Williamson ('77).

[2] Wild ('00).

tissues of the petiole of *Medullosa anglica*[1] as further evidence of the probable relationship of *Medullosa* and *Trigonocarpus*. As we shall show later on in this paper, the similarity is possibly not quite as close as Wild supposed.

The principal object of the present paper is to present the results of a detailed examination of a large number of sections of the common form *of Trigonocarpus* (*T. Parkinsoni*). Many of the sections and specimens are in Dr. Scott's collection, and others have been borrowed from various sources including the British Museum (Natural History), the Manchester Museum, the Newcastle Museum, the Cambridge University Collection, and that at University College, London. For facilities to examine these and other specimens we are indebted to Mr. E. A. Newell Arber, F.G.S., Mr. W. E. Hoyle, D.Sc., Prof. F. W. Oliver, F.R.S., Prof. M. C. Potter, F.L.S., Prof. A. C. Seward, F.R.S., and Dr. A. S. Woodward, F.R.S.

After describing the common form we shall briefly describe a new species obtained from a roof-nodule at Shore-Littleborough, Lancashire. The slides have been submitted to us for examination by Prof. F. W. Oliver, F.R.S., after whom we have named it *Trigonocarpus Oliveri*. A short account will then be given of some features of interest in connexion with the casts and impressions, and finally a second part of the paper will be devoted to a discussion of the general bearing of our work and the probable affinities of the seed.

III. Trigonocarpus Parkinsoni, Brongn.

General characters of the seed.

The seed as ordinarily preserved in the petrified condition is large, and of an oval form which is well shown in the longitudinal sections figured by Hooker and Binney[2] and by Williamson[3]. The general oval shape of the body of this seed as seen in the longitudinal sections is very characteristic, and different from that of the new species *Trigonocarpus Oliveri* (Pl. XIII, Fig. 19). The greatest width is about half-way up the body of the seed, i. e. of the rounded part excluding the micropylar beak. The transverse sections across the body of the seed show the three prominent ridges on which the generic name is based (see Pl. XI, Fig. 5). There is but little variation in size among the seeds which we have examined, and all appear to be in about the same stage of development. Some of the largest specimens which we have examined have a maximum diameter across the body of the seed (measuring to the outer margin of the hard testa only) of about 1·6 cm. The usual transverse diameter is less than this, about 1·3–1·4 cm. The length of the body of the seed, without the micropylar beak, is usually 2–2·5 cm.

[1] Scott ('99). [2] Hooker and Binney ('55). [3] Williamson ('77).

In many Palaeozoic seeds with somewhat Cycadean organization, the micropylar region is elongated into a beak-like structure. Ordinary longitudinal sections of *Trigonocarpus Parkinsoni* commonly exhibit some trace of the micropylar beak, but it is rarely, if ever, preserved throughout its length. In many of the impressions, such as those from Newcastle figured in this paper (Pl. XII, Figs. 16, 17, and Pl. XIII, Fig. 18), the beak is about equal in length to the body of the seed, but as the sections show that the beak at the top was very slender and devoid of hard tissue, it is probable that part of it has been lost. In an impression of *Trigonocarpus Parkinsoni* figured in a paper by one of us[1] the long micropylar tube is quite double the length of the body of the seed, but this may possibly represent a distinct species from those described in this paper. In longitudinal sections of the petrified seeds, the micropylar beak is generally quite short, probably owing in some cases to the slightly oblique character of the section. In many cases it seems probable that the greater part of this elongated structure was broken off or had decayed prior to fossilization, when the seeds formed part of the medley of vegetable débris of which the coal-balls are composed.

Taking the micropylar beak into account, the total length of a full-sized seed must have been at least 4–5 cm., and may have been greater than this.

There is some evidence that the seed was attached to its parent axis by means of a stalk (see Pl. XII, Fig. 12).

A common feature of many of these old seeds is the possession of a thick two-layered testa, of which the outer thinner-walled zone or *sarcotesta* is always partly or entirely destroyed. The inner layer, which we shall describe as the *sclerotesta* (=Williamson's endotesta), consists of thick-walled elements, many cells in thickness, and all filled with dark contents. The sclerotesta bears three prominent ridges on its outer surface (Pl. XI, Fig. 5, *s. t.*), with corresponding furrows on the inner side marking the position of the three sutures, along which the testa may have split open when the seed was ripe. In each space between two main or principal ridges, are two, or more commonly three, less prominent elevations which we shall describe as secondary ridges (Pl. XI, Fig. 5, *s. t.*), so that the whole number of longitudinal ridges down the body of the seed is usually twelve. The ridges along the body of the seed undergo changes in number, and in the relative prominence of the principal and secondary ridges when traced upwards into the micropylar region, as will be described in detail later on in this paper. The degree of prominence of the various ridges is subject to considerable variation, even in the body of the seed; in some specimens the secondary ridges are scarcely visible, and the sclerotesta consequently becomes only three-ridged.

[1] Scott ('05), (1) Fig. 33, p. 146.

The sclerotesta is in many cases the outermost tissue preserved, but in good specimens this is succeeded on the outside by a delicate, thinner-walled tissue, the sarcotesta (Pl. XI, Fig. 5, *sa*). This layer is never preserved in its entirety, owing to its weak construction and to its position on the outside of the seed, though parts of it may be perfect. Owing to the more or less collapsed condition of the sarcotesta, it is impossible to make an accurate estimate of its total thickness in the natural state, but it must certainly have exceeded 2 mm., and was probably considerably thicker than this. The sarcotesta is bounded on the exterior by the well-differentiated limiting layers to which Mr. Wild first directed attention[1], and traces of which are found in most of the best slides (see Pl. XI, Figs. 1, 3, 5, 6, 7, 8, *l.*, Pl. XII, Fig. 11, *h.* and *e.*, and Pl. XIV, Fig. 23).

The two layers of the testa, when traced upwards, are continued into, and form the walls of, the long micropylar beak to which we have already drawn attention.

The sclerotesta of the micropylar beak is, in its lower part, more or less triangular in transverse section (Pl. XI, Fig. 7, *s. t.*), and throughout the greater part of its length the beak possesses a triangular central tube or micropylar canal. Outside the sclerotesta of the beak is a great development of the sarcotesta, appearing, as seen in the transverse sections, as a wide flat wing on either side of the micropyle (see Pl. XI, Figs. 7, 8, and Pl. XII, Figs. 9, 10). The 'wing' is bounded externally by the usual limiting layers. The question as to how far the flattened, wing-like form is natural, and how far it is caused by loss of the internal tissue of the sarcotesta and flattening by pressure is difficult to answer, but will be discussed later in this paper. Within the testa of the body of the seed, and usually separated from it by a space, is the nucellus (Pl. XI, Fig. 5, *n.*). Longitudinal sections show the chalazal attachment of the nucellus at the base of the seed (Pl. XII, Fig. 12, *p.*). Above the chalazal attachment the nucellus appears to have been free from the integument, agreeing in this respect with *Stephanospermum*[2] and some other Palaeozoic seeds, and differing from the modern Cycads, in which only a comparatively small upper portion of the nucellus is free. This view of the relation of nucellus and integument has already been suggested by Professor F. W. Oliver for a French form, *Trigonocarpus pusillus*[3]. At the micropylar end of the nucellus is the pollen-chamber, long ago indicated in some of Hooker and Binney's figures, and described and more fully illustrated by Williamson. The pollen-chamber (Pl. XII, Fig. 13, *p. c.*) forms a wide dome 2·5–3 mm. in diameter at the base, and was provided at the top with a narrow beak or canal, *be.* not more than 300 μ in diameter (see also Pl. XII, Fig. 14). In the section figured the pollen-chamber beak is barely half a millimetre in length, but presumably it extended much farther in the natural condition.

[1] Wild ('00). [2] Oliver ('04) (1), p. 368. [3] Oliver ('04) (2), p. 99.

No pollen-grains are present in the pollen-chamber of any of the specimens which we have examined.

Within the nucellus, and separated from it by a space, is the megaspore membrane (Pl. XI, Fig. 5, *m.*), which in all our slides is preserved merely as a line without visible structure. No prothallium or archegonia are preserved.

The vascular supply to the seed consists of two sets of bundles, which are given off at different levels from the main bundle. The supply strand which enters the base of the seed, while still at a considerable distance below the base of the nucellus, gives off a number of bundles (probably six) which traverse the sarcotesta of the seed, lying opposite to some of the smaller (secondary) ridges of the sclerotesta. These sarcotestal bundles are embedded in the inner denser tissue of the sarcotesta, and were evidently complete bundles with phloem as well as xylem, although the former has not been preserved. From the best preserved examples it appears probable that the xylem was of mesarch structure, the smaller, protoxylem elements occupying an internal position.

The second vascular system of the seed runs in the nucellus and is rather complex in its arrangement. It springs from the upper end of the main bundle which spreads out in the lower part of the seed, and forms what appears to be a practically continuous sheath of tracheides, comparable with the tracheal mantle of a *Stephanospermum*[1]. Transverse sections across the central part of the body of the seed show that the tracheides range themselves in more or less definite and crowded longitudinal strands, which are connected by transverse anastomoses running in a tangential direction. The nucellar vascular system has been traced through the whole length of the nucellus almost to the base of the pollen-chamber, and indications of possible tracheides have been detected even in the beak of the pollen-chamber itself.

The Testa.

The Outer Coat or Sarcotesta. The outer thin-walled layer of the seed-coat is usually either destroyed altogether, or is represented by only a few layers of cells, immediately external to the dark-coloured inner layer or sclerotesta. Externally the cells representing the sarcotesta usually have no definite limiting layer, while internally the junction with the sclerotesta is a sharp one, especially in transverse sections across the body of the seed. In good specimens, however, the sarcotesta is better preserved, and is then found to be a tissue of considerable extent, limited externally by well-differentiated epidermal and hypodermal layers (Pl. XI, Figs. 5, 6). The sarcotesta is never completely preserved, but is always more or less collapsed and disorganized, so that it is not possible to estimate its original

[1] Oliver ('04) (1), p. 363.

thickness with certainty. In the transverse section of the central part of the body of the seed shown in Pl. XI, Fig. 5, the sarcotesta *sa* at its thickest part measures about 2 mm., and there is clear evidence that the outer portion, with the exception of the limiting layers *l*, has been destroyed, so that the total thickness would have been considerably more than this, at least in the spaces between the ridges of the sclerotesta where it was probably thickest.

In some cases, as in the transverse section figured (Pl. XI, Fig. 5), the external boundary-line of the sarcotesta follows fairly closely the contour of the sclerotesta within, but we are inclined to think that this correspondence is not natural, but is merely a result of more or less desiccation and compression prior to fossilization, and that the transverse section of the whole seed may have been circular. The correspondence in outline between the sarcotesta and sclerotesta is seen in several of Williamson's original slides figured in his paper, and his remark that: 'though the external outline of the sarcotesta is less sharply defined than that of the endotesta [=our sclerotesta], we yet see that to a considerable extent the former follows the latter, the differences seen in the sections being due to shrinkage from some collapse in the soft parenchymatous cells of the outermost layer,'[1] seems to imply that Williamson thought the correspondence was a natural one.

The sarcotesta appears to have been thinnest around the body of the seed, and to have increased in thickness above and below. This is shown in some of the sections figured in this paper. Pl. XI, Fig. 3, is a transverse section across the base of the same seed as that shown in Pl. XI, Fig. 5. The central mass of dark sclerotic tissue, *s. t.*, continuous with the sclerotesta of the body of the seed, is surrounded by a well-developed sarcotestal tissue, which in places is quite 3 mm. thick, and in its natural condition must have been much thicker than this, as a considerable loss of tissue is indicated by the remains of disorganized cells, as well as by the doubling in of the limiting layers of the sarcotesta, *l*. Pl. XI, Fig. 1, is another transverse section of the same seed, but taken still lower: the inner part of the sarcotesta is preserved, but great loss of tissue is shown by the limiting layers which are in places doubled in and repeated four or five times.

Passing to the upper end of the seed, a transverse section across the lower part of the micropylar beak is shown in Pl. XI, Fig. 7, and it will be seen that the sarcotesta *sa* is here quite well-developed, as indeed it continues to be right up to the end of this organ.

The sarcotesta consists typically of thin-walled parenchyma without special peculiarities. The inner portion is compact and consists of cells without visible inter-cellular spaces. In the body of the seed, these thin-

[1] Williamson ('77), p. 250.

walled cells abut quite suddenly on the dark-coloured thicker-walled elements of the sclerotesta (Pl. XI, Fig. 5). The outer portion of the sarcotesta appears to have been lacunar, which fact helps to account for the almost complete destruction of this tissue. The lacunar zone is occasionally preserved to a slight extent in the bays between the longitudinal ridges of the sclerotesta. Pl. XIV, Fig. 24, shows the lacunar sarcotesta in one of Williamson's original slides, and a similar tissue can be made out in several sections belonging to the same series.

It has been stated above that the sarcotesta is sharply delimited from the sclerotesta; the sudden transition is well seen in sections passing across the central part of the body of the seed, such as that shown in Pl. XI, Fig. 5. A section showing the details of the structure of the inner sarcotesta and outer sclerotesta, such as that figured in Williamson's paper [1], shows that the sharp demarcation is not simply the result of thickening of the walls of the elements of the sclerotesta, for the radially elongated cells of the outer part of the sclerotesta abut suddenly on the rounded cells of the sarcotesta, so that there is seen to be a difference in the form of the elements as well as in the thickening of the walls.

In transverse sections passing across the lower part of the seed, such as are shown in Pl. XI, Figs. 1 and 3, the transition from sclerotesta to sarcotesta is much more gradual. In Fig. 1 the sclerotic tissue is seen surrounding a triangular space *b* enclosing the incoming vascular bundle: this dense tissue passes over quite gradually into the surrounding thin-walled sarcotesta. In Fig. 3, which is cut at a somewhat higher level across the same seed, the outermost sarcotestal cells are seen to be quite thin-walled, but nearer to the central sclerotesta *s. t.* the walls become thicker and the transition is more or less gradual.

Sections across the upper part of the seed also show a more or less gradual passage between the two layers, as will be seen by examining Pl. XI, Figs. 7 and 8, and Pl. XII, Fig. 9, which are transverse sections cut at successively higher levels across the micropyle.

The absence in certain regions of sharp demarcation between the two layers of the testa is of interest in the comparison of *Trigonocarpus* with the seeds of *Cycas* and other recent forms of similar organization. Miss Stopes has shown that it is usually difficult to draw a boundary line between the outer flesh and the stone layers in a modern Cycadean seed, and she insists that the stone layers, or at least the outer part of them, belong morphologically to the outer flesh and so form one layer, not two [2]. We shall leave the further discussion of the bearing of this question of the single or double nature of the Cycadean integument, and the attempt to explain the complicated integument of a Cycad ovule as the morphological equivalent of a *Lagenostoma* with the cupule and integument fused together,

[1] Williamson ('77), Pl. 14, Fig. 112.

[2] Stopes ('05), p. 562.

to the concluding part of this paper. The relation of the sarcotesta and sclerotesta in *Trigonocarpus* is such as to indicate that they are morphologically one integument, just as is the case in the structurally very similar layers of a modern Cycad seed.

Other Palaeozoic seeds also agree in this respect. *Polylophospermum stephanense* (Brongn.), a radiospermic seed from Grand'Croix which agrees with *Trigonocarpus* in important characters, has been shown by Professor Oliver to exhibit gradual transition from the hard sclerotesta to the softer enveloping sarcotesta [1].

The sarcotesta is bounded externally by the definite limiting layers to which Wild first directed attention (p. 95). The figure given by Wild [2] shows an apparent epidermis consisting of closely set radially elongated dark-coloured cells forming a kind of palisade tissue, usually only one, but sometimes two, cells in thickness. These palisade cells are covered by what is described as a thin cuticle. From the resemblance of this tissue in *Trigonocarpus* to the limiting layer of the petioles of *Medullosa anglica*, in which the epidermis has a well-marked palisade form [3], as well as from the constant association of these two forms, Wild was led to believe that *Trigonocarpus* is probably the seed of *Medullosa*. Re-examination of some of Wild's slides shows that some of them exhibit traces of a layer of small cells outside the palisade cells, and forming a true epidermis to them. The epidermal cells are better seen in some other slides, especially in some of those obtained from the Manchester Museum. The limiting layers of the sarcotesta is shown in Pl. XIV, Fig. 23, drawn from one of the Manchester slides, and magnified about 40 times. The epidermis is shown at *e*, and the hypodermis of palisade-like cells at *h*. The epidermal cells are quite small and apparently empty; their outer walls are thickened to form a continuous cuticle which is frequently preserved as a detached line, when the radial walls of the epidermal cells are wholly destroyed. The detached cuticle of the epidermis is visible in parts of Pl. XI, Fig. 1, as well as in some of the other photographs in Plates XI and XII. Occasionally the epidermal cells are visible in surface view as a simple layer of small isodiametric cells. The hypodermis consists of much larger cells usually more or less radially elongated and sometimes very irregular in shape (Pl. XIV, Fig. 23); sometimes only one elongated cell appears, elsewhere the place of a single elongated cell is taken by a row of two or three short cells. All the cells of the hypodermis agree in being filled with dark-coloured contents which give to the layer as a whole a very characteristic appearance much resembling that of the outer tissues of a *Medullosa* petiole. Re-examination of the *Medullosa anglica* slides has failed to detect any trace of an epidermis outside the rarely preserved palisade tissue which apparently includes the real epidermis of this form. At the same time, in

[1] Oliver ('04) (1), p. 101. [2] Wild ('00), Fig. 14. [3] Scott ('99), Pl. 11, Fig. 13.

view of the strong probability, on other grounds, of a relationship between *Medullosa* and *Trigonocarpus*, it is not impossible that a small-celled epidermis may be subsequently discovered in the former genus. In the meantime, the apparent absence of this layer in *Medullosa* renders Wild's comparison less close than he thought.

As already briefly described, the outer series of vascular bundles of the seed traverse the sarcotesta and they are occasionally seen in transverse sections of the seed, although, owing to the usual non-preservation of anything more than a few layers of the inner sarcotesta, such sections are quite rare. The bundles spring from the main supply-strand at a considerable distance below the chalaza, and bending outwards traverse the sarcotesta of the body of the seed, running opposite to the secondary ridges of the sclerotesta. The position of one of the sarcotestal bundles is indicated at *s. b.* in Pl. XI, Fig. 5. The bundle itself cannot be distinguished in this photograph, but is shown more highly magnified in Pl. XIV, Fig. 28. Traces of sarcotestal bundles can be seen in other slides, and in all cases they appear opposite to the secondary, never to the principal, ridges of the sclerotesta. The total number of sarcotestal bundles would thus have been nine, or perhaps only six, in ordinary specimens, such as that shown in Pl. XI, Fig. 5. It seems probable that the number was really six. In no single section are sarcotestal bundles seen opposite to all the secondary ridges of the sclerotesta, and in all the sections in which the bundles are visible, they appear invariably opposite to the two outer of each set of three secondary ridges, never to the central one. The central secondary ridge frequently differs from the lateral ones in being more rounded, and, moreover, as we shall see later, it is the first ridge to disappear when traced up to the upper end of the seed. It seems probable that it also differs in having no sarcotestal bundle opposite to it. That there are six, not nine, sarcotestal bundles is also favoured by examination of the section across the base of the seed shown in Pl. XI, Fig. 3. This is an approximately transverse section cut some distance below the chalaza; careful examination of the original section shows six bundles, *s. b.*, embedded in the compact sarcotesta, and doubtless these are the same bundles that afterwards appear opposite to some of the secondary ridges of the sclerotesta in the body of the seed. The central mass of sclerotic tissue which is seen in this section (Pl. XI, Fig. 3, *s. t.*) is produced on one side into a projection, which in all probability represents the lower end of one of the principal longitudinal ridges of the sclerotesta, cut through owing to slight obliquity of the plane of section. No sarcotestal bundle can be seen opposite to this ridge, although two occur symmetrically disposed on either side of it, so that this section helps to confirm the conclusion as to the number and position of the bundles arrived at above.

An individual sarcotestal bundle is shown, magnified about 200 times, in Pl. XIV, Fig. 28. The position of this bundle is shown at *s.b.* in Pl. XI, Fig. 5. These bundles are quite small; the total radial diameter of the one figured cannot have been greater than ·15 mm., and the diameter of the largest tracheide about ·025 mm. The bundle is only imperfectly preserved, and no trace of phloem elements are visible. The tracheides *t* are nearly in contact with the ordinary parenchyma of the sarcotesta *sa* on the inner side of the bundle, while on the outer side there is a space which is partly occupied by decomposed tissue *d. t.*, which probably represents the original phloem elements. The bundle was therefore probably a collateral one with external phloem. There is some evidence that the xylem was mesarch in structure, as the smaller elements, presumably protoxylem tracheides, occupy a more or less central position. This conclusion is also favoured by the examination of longitudinal sections, as will be explained later (p. 115). This bundle is separated from the sclerotesta of the secondary ridge, to which it runs parallel, by sarcotestal tissue about ·3 mm. in thickness. The longitudinal sections are not sufficiently good to enable us to trace the sarcotestal bundles upwards to their termination. The transverse section shown in Pl. XI, Fig. 5, cannot be far removed from the middle of the body of the seed, and Pl. XI, Figs. 6–8, and Pl. XII, Figs. 9, 10, are cut at successively higher levels. In all these sections the inner sarcotesta is fairly well preserved, and it seems likely that if vascular bundles were present they would have been preserved also. No trace of such bundles is visible, so that it seems probable that they did not extend farther up than the body of the seed.

As *Trigonocarpus Parkinsoni* is the first of the Cycad-like seeds of the Carboniferous Period, the Stephanospermeae of Professor Oliver, in which it has been possible to give a fairly complete account of the sarcotesta, it will be useful to compare this layer with what is known of the corresponding tissue of other related fossil seeds, and with the 'outer flesh' of recent Cycadean seeds.

The possession of an outer soft layer to the seed is believed to be a common character of all the seeds which constitute Professor Oliver's Stephanospermum-Group of Radiosperms, although in the type genus, *Stephanospermum*, evidence for its existence, other than from analogy, is absent[1]. In most of the genera included in this group, *Aetheotesta*, *Trigonocarpus*, *Tripterospermum*, *Polylophospermum*, *Codonospermum*, &c., an outer fleshy, or sometimes fibrous, sarcotesta has been proved to exist, although it is usually very badly preserved.

Trigonocarpus pusillus, Brongn., a small representative of the genus from the French deposits, recently re-described by Professor Oliver, possesses a sarcotesta of two or three layers of thin-walled, iso-diametric,

[1] Oliver ('04) (1).

parenchymatous cells which probably represent but a portion of a more extensive tissue[1].

The detailed structure of the seeds of the Cycads has been recently described by Miss Stopes[2]. The 'outer flesh' or sarcotesta, which is universally present, is composed of parenchyma with numerous gum canals and tannin sacs and is bounded externally by an epidermis of radially elongated cells. The sarcotesta is traversed by a series of vascular bundles which spring from the central supply bundle entering at the base of the seed. In position these bundles lie opposite to the longitudinal ridges of the underlying 'stone' or sclerotesta as has been described above in *Trigonocarpus*. In the Cycads the number of sarcotestal bundles always corresponds exactly with the number of ridges, so that in *Macrozamia spiralis* where the stone is provided with twelve well-marked ribs there are also twelve bundles, while the bilateral seed of a *Cycas* which possesses only two ridges has only two bundles, although each bundle contains two vascular strands in some species. As described above, in *Trigonocarpus* it is probable that the sarcotestal bundles occur opposite to certain of the longitudinal ridges only, not to all.

Returning to the Cycads the individual sarcotestal bundles are described as collateral with mesarch xylem. In some forms, such as *Cycas circinalis*, the bundles are of a simple type with external phloem and internal xylem provided with a considerable development of transfusion-tracheides, the whole bundle being surrounded by a sheath composed of peculiar cells provided with reticulated lignified thickenings[3].

In some other forms the sarcotestal strands are more complex in structure although their position remains the same. In *Cycas media* each strand consists of two collateral bundles with their phloems directed towards one another, so that the outer bundle of the two has, of course, reversed orientation. The xylem of each bundle is mesarch, and the inner one of the two possesses a considerable development of transfusion elements[4]. In *Cycas revoluta* the bundles run in pairs through the outer flesh.

In yet other species the structure becomes still more complex, for in *Cycas Beddomii* the whole strand, consisting of two bundles embedded in fine conjunctive tissue containing many transfusion elements, is surrounded by a well-differentiated sheath composed of the peculiar cells mentioned above in *Cycas circinalis*[5].

As far as can be judged from the transverse sections of imperfectly preserved sarcotestal bundles around the body of the seed such as that shown in Pl. XIV, Fig. 28, *Trigonocarpus Parkinsoni* conforms to the simplest of these types, that of *Cycas circinalis*, but owing to the imperfect

[1] Oliver ('04) (2), p. 98. [2] Stopes ('04). [3] Stopes ('04), p. 440.
[4] Stopes ('04), p. 442. [5] Stopes ('04), p. 445, Fig. 8.

preservation the evidence is not conclusive. More perfectly preserved sarcotestal bundles at the base of the seed afford evidence of a more complex structure which may be compared with that of *Cycas Beddomii*, as will be explained in detail in our description of the lower end of the seed (p. 115).

The Sclerotesta. We may now consider some of the details of the hard layer of the testa, the sclerotesta, which lies within the sclerotesta. The structure of the sclerotesta has been previously described in some detail by Hooker and Binney[1], and later by Williamson[2], and as far as internal structure is concerned we have but little to add to their descriptions. This hard layer forms a complete investment to the seed excepting only at the upper end of the long micropylar beak, where it is, of course, perforated by the opening of the micropylar canal. Its thickness, when measured between the ridges of the body of the seed, in such a section as that shown in Pl. XI, Fig. 5, *s. t.*, is 1–1·5 mm. Most longitudinal sections show a thickening of the sclerotesta as it is traced from the chalaza upwards to the base of the micropylar beak. This may also be seen in some of the transverse sections, as by comparing Pl. XI, Fig. 5, and Pl. XI, Fig. 7, the former cut across the central part of the body of the seed, and the latter across the lower part of the micropylar beak, although as the two sections are not cut from the same seed the comparison as to thickness must not be carried too far. Comparison may also be made of Pl. XII, Fig. 12 and Fig. 13, which are longitudinal sections of the lower part, and of the upper part respectively, of two seeds; in Fig. 12 the thickness of the sclerotesta is at its thinnest part about ·8 mm., while in Fig. 13 the maximum thickness is 2 mm. The sclerotesta of the lower part of the same seed as that figured in Pl. XII, Fig. 13, has a thickness of only 1 mm.

As already briefly described the sclerotesta has a very characteristic form when seen in transverse sections. The normal number of ribs projecting on the outer side is probably always twelve in sections cut across the body of the seed, such as Pl. XI, Fig. 5. The twelve ribs are usually easily distinguishable into two sets, the principal ribs (Pl. XI, Fig. 5, *s'. t'.*) always three in number, and the smaller or secondary ridges (Pl. XI, Fig. 5, *s. t.*) usually nine in number. The degree of prominence of the principal ridges is subject to considerable variation in different seeds as well as at different levels of the same seed. In most specimens the principal ridges are readily distinguished from the secondary ones by their greater prominence as shown in Pl. XI, Figs. 5, 6. In some other seeds the principal ridges are broader and less pointed, and in yet other cases there is only a slight difference in size and shape between these and the secondary ridges.

[1] Hooker and Binney ('55).

[2] Williamson ('77), p. 252.

In many transverse sections one or more of the principal ridges shows a central crack as though the seed naturally split open along these sutures (see Pl. XI, Figs. 5, 6). This splitting is never seen in relation to the secondary ribs.

The principal ridges when traced from the centre of the body of the seed upwards to and along the micropylar beak gradually become less prominent and ultimately disappear. This change will be seen by examining the series of transverse sections represented in Pl. XI, Figs. 5–8. Fig. 5 is cut near the centre of the body of the seed and exhibits what may be called the normal arrangement, the principal ridges being easily distinguished by their size. In Fig. 6, which is cut at a somewhat higher level than Fig. 5, these ridges are still sufficiently large to be readily identified even without the characteristic splitting which is well shown in this slide. In Fig. 7, which is cut across the lower part of the micropylar beak the principal ridges of the body of the seed appear opposite to the angles of the triangular micropylar tube and although still large, some of the secondary ridges have become equal to them. Lastly, in Fig. 8, which is cut higher up the micropylar beak, the principal ridges lying opposite to the points of the micropylar canal have practically disappeared altogether.

Passing to the secondary ridges, these are also found to vary considerably in different specimens and at different levels. In a typical example, a section across the central part of the body of the seed, such as Pl. XI, Fig. 5, shows the three secondary ridges, *s. r.*, lying between two principal ribs and about equally developed. In some cases, however, the median ridge of each set of three is so slightly developed as to be practically absent, so reducing the total number of ridges (principal and secondary) to nine, while in yet other examples all the secondary ridges are ill-developed so that only the three principal ridges are at all conspicuous. Changes also take place in the secondary ridges when they are traced upwards as will be seen by again examining the series of transverse sections at various levels (Pl. XI, Figs. 5–8). In Fig. 5 all the secondary ridges are about equally developed; in Fig. 6 which is cut at a higher level the central ridge of each set of three has 'flattened out' so that it has nearly disappeared while the two lateral secondary ridges are equally prominent; in the still higher sections while the lateral secondary ridges still remain conspicuous the central one is unrepresented. Hence the most persistent longitudinal ridges appear to be the lateral secondary ribs, or those to which the sarcotestal bundles are probably opposite, as we have previously shown.

A knowledge of these regional variations in the ridges of the sclerotesta is of some importance in the attempt to interpret the structures seen in casts, as will be explained later.

On the inner side of the sclerotesta there are three furrows corresponding in position with the three principal external ridges. These furrows vary in depth in different parts of the seed, as is well seen in transverse sections. In sections cut across the lower part of the body of the seed, the grooves are very slightly developed, and are sometimes absent altogether, while in sections taken at successively higher levels, the furrows become more and more pronounced. Comparison of Pl. XI, Figs. 5–8, will serve to demonstrate this fact, as also that the points of the triangular micropylar tube are the upward prolongations of the three shallow grooves seen in sections across the body of the seed such as shown in Fig. 5. The three grooves on the inner side of the sclerotesta, of course account for the three ridges which are shown on the surface of the common internal casts of the cavity of the seed.

With regard to the minute structure of the sclerotesta, we have little to add to former descriptions. In good thin sections the sclerotesta is readily distinguishable into two regions, an inner zone (Pl. XI, Fig. 6, *s. t.*), which is very dark in colour, and consists mainly of elements which are elongated in the vertical direction, and an outer zone (Fig. 6, *s'. t'.*) in which, especially in sections cut across the body of the seed, many of the cells are seen to be elongated in a horizontal radial direction, and so form a kind of outer palisade layer to the sclerotesta. This is best shown, in Fig. 6, in the sclerotesta of the secondary ridges. If the section is a thicker one, the distinction of the sclerotesta into two zones is not so clearly seen. In more detail the structure of the sclerotesta of the central part of the body of the seed is as follows. Starting from within, and considering the region between the longitudinal ridges, we have first about four to six rows of small elongated elements running in the longitudinal direction, and filled with the dark-coloured material which is characteristic of all the elements of the sclerotesta. These cells are thick-walled and fairly uniform in size, many times longer than broad, and of prosenchymatous form. Hooker and Binney describe these cells as forming a lining of long slender tubes to the whole body of the fruit (i. e. seed), and assert that among these cells some may be found marked with annular or spiral bands[1]. Williamson states that traces of the same structure appear in his sections[2]. We have examined a considerable number of longitudinal sections of *Trigonocarpus*, including the original Williamson and Hooker and Binney slides, but we have been unable to confirm this observation.

Outside these longitudinally running cells are seen some rows of elongated elements which run in a horizontal direction around the body of the seed, and outside these is a zone in which the cells appear to meander irregularly, the elements passing across one another in all directions. In a tangential section of this part of the testa[3], the elongated cells are seen

[1] Hooker and Binney ('55). [2] Williamson ('77), p. 252. [3] Slide 944 S.

running in bundles which interweave with one another almost like basket-work. These cells are followed by the layer of radially elongated elements or palisade layer which usually constitutes the greater part of the thickness of the sclerotesta, and forms its outermost part. The actual proportion in thickness of the palisade layer to the rest of the sclerotesta is subject to considerable variation in different seeds. Many of the details of the structure of the sclerotesta are shown in Williamson's figures (Williamson, 1877, Pl. 14, Figs. 112, &c.). At the principal ridges the structure is somewhat different from that described above, as the majority of the cells follow a mainly longitudinal course, so that they are cut across in transverse sections of the seed. Many slides show dark-coloured elongated cells, passing radially through the centre of each principal ridge, and it is along these elements that the splitting which is so frequently seen takes place.

The general plan of structure as given above, appears to have been the usual one in the sclerotesta of the Palaeozoic Cycad-like seeds, and a similar structure has been described in detail by Prof. Oliver in *Stephanospermum akenioides*, Brongn., and *S. caryoides*, Oliver[1]. It also appears to agree in a general way with the structure of the stony layers of the seed of a modern Cycad. Miss Stopes states that: 'It is very clearly marked in many Cycads that the stone has at least two layers, an inner one of mainly vertically running stone cells, as in the integument of *Lagenostoma ovoides*, and an outer one of mainly horizontally running stone cells.'[2] This layer is surrounded by the usually undifferentiated cells of the outer flesh. In some Cycads the structure of the outer layers of the testa is much more complex, as in *Cycas Beddomii*, described by Miss Stopes[3]. In this form the stony layer (sclerotesta) and outer flesh exhibit the following details of structure. The innermost part of the stone is formed of layers of vertically elongated sclerotic cells comparable with the inner zone of *Trigonocarpus Parkinsoni* as described above. These are succeeded on the outer side by a number of layers of elongated sclerotic cells which follow a horizontal direction and run circularly round the seed. A similar zone is found in *Trigonocarpus*. Next follows a thick layer of radially elongated horizontal cells, which are described as belonging to the outer fleshy layer. These cells have somewhat thickened slightly lignified walls, and may be compared in a general way with the outer layer or palisade-layer of the *sclerotesta* of *Trigonocarpus*. The radially elongated cells pass externally into others with reticulately thickened walls, and these again into the ordinary tissue of the outer flesh, consisting of large parenchymatous cells with pitted cellulose walls similar to the ordinary sarcotestal cells of *Trigonocarpus*. The reticulately thickened tracheide-like cells, which occur between the radially elongated inner cells and the ordinary parenchymatous

[1] Oliver ('04) (1), p. 364 and p. 373. [2] Stopes ('05), p. 562.
[3] Stopes ('04), p. 444 and Fig. 7.

outer cells of the sarcotesta in *Cycas Beddomii* are described as having a water-storing function, and their presence is perhaps to be correlated with the dry habitat of this species[1]. No similar cells are found in *Trigonocarpus*. Apart from this difference it will be seen that in the details of structure of the testa, *Trigonocarpus Parkinsoni* exhibits somewhat close similarity with that of one of the most complicated of modern Cycads.

The Inner Flesh. The next question which may be considered is that of the possible existence of a thin-walled tissue within the sclerotesta, and corresponding in position with the 'inner flesh' of the seed of a Cycad. Such a layer may be called an 'endotesta,' but in view of the use of this term by Williamson and others for the stony layer or sclerotesta of *Trigonocarpus*, we have thought it better to avoid confusion by using the term 'inner flesh.'

In none of our specimens is such a layer preserved in a recognizable condition, excepting just at the base of the body of the seed. In one of our slides[2], a longitudinal section passing in a slightly tangential direction through the base of the seed, a tissue consisting of parenchymatous cells, is seen lying between the nucellus and the sclerotesta. The only evidence for the existence of an inner flesh higher up in the body of the seed is found in the commonly somewhat irregular and 'unfinished' appearance of the inner boundary of the sclerotesta. This 'unfinished' appearance is particularly noticeable at the upper part of the body of the seed, and the lower part of the micropylar tube. On the whole we think the evidence is sufficient to make it probable that *Trigonocarpus* possessed a continuous inner fleshy layer within the sclerotesta. The reason why the cells of this layer are not more generally preserved, is probably that the cells themselves were of a delicate nature, and also that the hard impermeable coat of the seed prevented the infiltration of the petrifying material before the tissue had undergone disorganization.

An inner fleshy layer probably existed in most of the Palaeozoic seeds of similar organization to *Trigonocarpus*, although it is never found even in a fair state of preservation. Prof. Oliver has described traces of such a tissue in *Stephanospermum caryoides*[3], while in *Stephanospermum akenioides* a delicate network of thin-walled cells is sometimes seen lying in the angle between the base of the nucellus and the lining of the sclerotesta[4], just as in some of our slides of *Trigonocarpus Parkinsoni*.

The very imperfect preservation of this tissue is unfortunate, in view of the great importance of determining its relation to the nucellus within, whether the nucellus stood up freely, separated from the testa throughout the greater part of its length, i. e. from the pollen-chamber to the chalazal

[1] Stopes ('05), p. 445.
[2] Slide 941 S.
[3] Oliver ('04) (1), p. 374.
[4] Oliver ('04) (1), p. 367.

attachment, as in *Lepidocarpon*, and, according to Prof. Oliver, probably in *Stephanospermum*[1], or whether the relations were as in the modern Cycads, and in *Lagenostoma*, in which the testa and nucellus are adherent excepting right at the top of the seed. A discussion of this question will best be deferred until after the structure of the nucellus has been described, but here we may say that in *Trigonocarpus* as in *Stephanospermum*, the evidence supports the view that the nucellus was free.

The Chalazal End of the Seed.

An account may now be given of some of the details of the structure of the lower part of the seed. Some of the longitudinal sections afford evidence that the seed was attached to the parent plant by means of a stalk. The stalk is shown in Pl. XII, Fig. 12. As shown in the figure, the sarcotesta of the stalk of this specimen has undergone considerable displacement and compression as is made evident by the crushed tissue on the right-hand side of the stalk and the repetition of the limiting tissues of the sarcotesta on the other. The form of the stalk shown in this section is therefore not the natural one, nor is the unsymmetrical position of the stalk with reference to the chalazal attachment above. Other sections show the stalk symmetrically placed. As shown in Pl. XII, Fig. 12, as well as in some other slides, the stalk appears to consist almost entirely of parenchymatous tissue continuous with the inner, denser, zone of the sarcotesta, but this appearance is really due to the fact that the section passes somewhat tangentially, not medianly, through the base of the seed, although, as it passes through the chalazal attachment of the nucellus it cannot be very far from the median plane. Apparently the median plane of the stalk is more closely approached right at the bottom of this section, as here it passes into sclerotic tissue, *s. t.*, which is probably part of the central core of the stalk continuous with the sclerotesta of the body of the seed.

Many of the more nearly median longitudinal sections show the central core of sclerotic tissue of the stalk in the form of a downwardly directed pointed prolongation of the sclerotesta, the shortness of which, in most of the slides, is doubtless due to the slight obliquity of the sections. In one section the sclerotic stalk has a length of about 1·5 mm., and in another slide it is traversed longitudinally by the main supply bundle of the seed. The sclerotic tissue of the stalk probably gradually died out below, the hard core becoming smaller as its tissue was replaced by the thinner-walled cells of the sarcotesta. We have already seen that in this part of the seed the passage from sclerotesta to sarcotesta is a gradual one. Judging from the longitudinal section shown in Pl. XII, Fig. 12, the sclerotic axis of the stalk must have had a length of more than 4 mm., as the sclerotic tissue, *s. t.*,

[1] Oliver ('04) (1), p. 368.

is at about that distance from the base of the sclerotesta of the body of the seed.

Passing to the transverse sections of the base of the seed we have in Pl. XI, Fig. 1, a section which is doubtless cut across the lower part of the stalk, probably about on a level with its lower end, as shown in the longitudinal section, Pl. XII, Fig. 12. In the section shown in Pl. XI, Fig. 1, the whole of the outer portion of the sarcotesta has been destroyed with the exception of the characteristic outer limiting layers, *l.*, which have evidently suffered much displacement by compression from without, as they are, in places, repeated as many as four times. The tissue preserved is mainly composed of thin-walled cells which pass gradually into the sclerotic tissue which surrounds the triangular space, *b.*, containing the entering vascular bundle. No trace of bundles or tracheides can be seen in the sarcotesta of this slide, probably because the section is just below the level at which the sarcotestal bundles are given off from the main bundle. The triangular form of the space in which the main bundle is contained is probably of some significance, especially as this form is much less marked in sections across the upper part of the stalk, such as the one shown in Pl. XI, Figs. 3 and 4. There is evidence from the longitudinal sections that the sarcotestal bundles were given off from the main bundle at a considerable distance below the base of the body of the seed, although, unfortunately, no section shows the actual branching. In the section figured in Pl. XII, Fig. 12, sarcotestal bundles can be seen in the slide at the points marked *s. b.*, *s. b.*, and one of these is figured in detail in Pl. XIV, Fig. 25, which will be described later. From the position of these bundles in the stalk it is clear that they must have arisen some distance below the level at which they are seen in Pl. XII, Fig. 12, so that the junction with the main bundle must have been at least 4 mm. below the base of the nucellus.

It is probable that the section which is shown in Pl. XI, Fig. 1, is cut quite near to the level at which the sarcotestal bundles are given off, and that the triangular form of the space is to be correlated with this fact. The six sarcotestal bundles may have been given off in pairs corresponding in position with the angles of the space, or they may have been only three in number, each afterwards dividing into two. That the section is below rather than above the plane of branching seems evident from the entire absence of sarcotestal bundles, since the preservation of the inner tissues of the testa is so good that they would almost certainly be seen if they had been present.

Pl. XI, Fig. 2, shows the central part of Fig. 1, magnified about 100 times. The xylem of the main supply bundle is preserved (*b.*), and has a width in the longest direction of ·28 mm. The surrounding thinner-walled tissue has been entirely destroyed, but presumably it was a similar

parenchyma to that seen surrounding the bundle in the higher section shown in Pl. XI, Fig. 4, *a*.

Pl. XI, Fig. 3, is a transverse section probably cut quite close to the base of the body of the seed, or across the upper part of the stalk, of the same specimen as that illustrated in Figs. 1 and 2. The central core of sclerotic tissue, *s.t.*, continuous with the hard coat of the body of the seed, is well seen, as also the gradual transition of this tissue into the surrounding sarcotesta with the characteristic bounding layers, *l.* The main vascular bundle, *b.*, is just discernible occupying a sub-central position in the sclerotesta, while in the surrounding sarcotesta six bundles, *s.b.*, can be seen. The outer part of the sarcotesta has been destroyed, with the exception of the external layers, *l.*, which are pressed in on the more durable tissues.

The central sclerotic tissue is produced on one side, probably owing to the section being somewhat oblique, so that it passes across the lower portion of one of the principal longitudinal ridges of the body of the seed. As before mentioned no sarcotestal bundle is seen opposite to the point of this projection, although two are seen occupying a fairly symmetrical position opposite to its sides. The bundle and surrounding tissues of Pl. XI, Fig. 3, are represented magnified about 100 times in Pl. XI, Fig. 4. The magnification of Figs. 2 and 4 is equal, so that the bundle at the two levels may be directly compared. In Fig. 4 the main supply bundle, *b.*, is shown, and appears to have a diameter of about ·14 mm., or is about half the size of the bundle of Fig. 2 cut at the lower level. The larger size of the bundle in Fig. 2 favours the view which has been already expressed, that in this section the sarcotestal bundles had not yet been given off, as the section is below the level of branching.

Returning to the section shown in Fig. 4, no clear evidence of a differentiated phloem surrounding the xylem can be made out, but the bundle is embedded in a comparatively large-celled, apparently parenchymatous tissue, which occupies the space within the core of hard tissue. It will be noticed that the triangular form is still preserved to a certain extent, though it is not as sharply marked as in the lower section (Pl. XI, Fig. 2).

In all probability the main supply bundle was concentric in structure. A definite protoxylem cannot be made out in this transverse section, and, unfortunately, the longitudinal sections which show the main bundle are not sufficiently good to help us in this matter. Miss Stopes has shown that in Cycads: 'The main supply bundle is usually either completely concentric or has a strong tendency in that direction; the bundles given off to the outer flesh are collateral and orientated with the phloem outwards, and with a considerable development of centripetal xylem; the strand continuing to supply the inner system has a strong concentric tendency[1].' In

[1] Stopes ('05), p. 560.

all probability *Trigonocarpus Parkinsoni* agreed with the modern Cycads in all these respects.

After giving off the sarcotestal strands, the main bundle, accompanied by its parenchyma, continues unbranched through the stalk of the seed, until it passes into the nucellus and gives rise to the inner vascular system to be described later. At the bottom of the body of the seed a pad of parenchymatous tissue is frequently seen between the nucellus and the sclerotesta; this tissue is doubtless continuous with the tissue which surrounds the vascular bundle of the stalk. Laterally this tissue may have been continuous with the presumed 'inner flesh,' or it may have narrowed down beyond the limits of the tracheal disk and become continuous with the hypoderm of the nucellus. On analogy with *Stephanospermum*[1] the latter view would appear to be probably the correct one.

In Pl. XII, Fig. 12, the position of some sarcotestal bundles is indicated at *s.b.*, *s.b.*, and in Pl. XIV, Fig. 25, this portion of the seed is shown magnified about eighty times. The tissues are very much disturbed, although the preservation of some of the elements is excellent. Parts of at least two and probably three sarcotestal bundles appear to be represented at *b.*, *b.*, *b.* In places the dark-coloured hypodermal cells of the sarcotesta, *h.*, have been pushed inwards until they are quite close to the bundles. Immediately surrounding the bundle on the right are the remains of a delicate cell-reticulum representing the tissue in which the bundles were embedded. Bounding the space in which the bundles are contained are some of the ordinary cells of the sarcotesta, *sa.*

Scattered about in the fine tissue which separates the bundles are numerous short tracheides or transfusion elements, *s.t.*, which remind one forcibly of the very similar elements contained in the fine conjunctive tissue surrounding the pairs of sarcotestal bundles in *Cycas Beddomii*. In this Cycad the two bundles of the outer flesh are complex in structure. Each is really double, and consists of two complete collateral mesarch bundles orientated with their phloems towards one another. The bundles are surrounded by a considerable development of very fine conjunctive tissue containing numerous transfusion tracheides with thickened walls and fine reticulate or spiral markings, and the whole is enclosed in a common sheath of relatively large cells with reticulately thickened lignified walls (see Miss Stopes' paper, 1904, Fig. 8, p. 445).

In *Trigonocarpus Parkinsoni* the sarcotestal bundles of the body of the seed are always very imperfectly preserved, but as far as the evidence goes a simpler type of bundle, such as is found in *Cycas circinalis*[2], is indicated, rather than the complex one of *Cycas Beddomii*. In the longitudinal section shown in Pl. XII, Fig. 12, and Pl. XIV, Fig. 25, however, a more complex type of bundle may be represented. Comparing our Pl. XIV, Fig. 25, with

[1] Oliver ('04) (1), p. 367 and Pl. 43, Fig. 21.

[2] Stopes ('04), p. 439.

Miss Stopes' figure, we see in *Trigonocarpus* two, or possibly three, bundles, *b.*, *b.*, *b.*, enclosed in a space bounded by cells of the sarcotesta, some of which, *a.*, show fine spiral markings, and perhaps indicate a sheath somewhat similar to that of *Cycas Beddomii.* In the fine tissue around and between the bundles are seen abundant fine transfusion elements or short tracheides, *s.t.*, very similar to those of this species of *Cycas.* It would thus appear that the sarcotestal bundles of *Trigonocarpus Parkinsoni*, at least in the lower part of the seed, were of a complex type.

Leaving the general structure of the bundle we may next consider some points of detail. In Pl. XIV, Fig. 25, one of the bundles (the upper one) is traversed for a distance in a longitudinal direction, while the other (below) is cut more obliquely. The upper bundle affords evidence of being mesarch in structure, as the elements with more diffuse thickening bands occupy a more or less central position. It thus confirms the conclusion arrived at from the transverse sections (p. 104). The commonest type of tracheide in the bundle is apparently thickened in a very fine spiral, whilst others show loose or compact reticulate markings. Some of the tracheides are shown more highly magnified in Pl. XIV, Fig. 26. The commonest type is that shown at A with exceedingly fine spiral or scalariform markings. A distinctly scalariform tracheide is shown at D with the same exceedingly delicate thickenings. As supporting the probable connexion of *Trigonocarpus* with *Medullosa* it is interesting to note the similarity of these tracheides of *Trigonocarpus* with the fine scalariform elements characteristic of the primary tracheides of *Medullosa anglica* [1]. Pl. XIV, Fig. 26, B, shows one of the tracheides from the central part of a bundle, perhaps one of the elements of the protoxylem, while C shows a common type in which two crossing series of spiral bands are present. Similar tracheides to the latter are common in *Medullosa anglica,* and, as the two series of thickenings are not quite equally in focus at the same time, they probably represent the markings of two adjacent tracheides on the common wall between.

Pl. XIV, Fig. 27, shows two of the short tracheides or transfusion elements of Pl. XIV, Fig. 25, *s.t.* Like the most typical tracheides of the bundles their markings are of a particularly fine character.

The Micropylar End of the Seed. The Question of a Wing.

A remarkable feature of this seed is the great prolongation of the micropylar tube, which in some specimens considerably exceeds the body of the seed in length. In the specimens figured in this paper, Pl. XII, Figs. 16 and 17, and Pl. XIII, Fig. 18, the micropyle is only slightly longer than the body of the seed, but it is possible, and indeed probable, that the full length of the beak is not preserved, although Fig. 17 has the appearance of being complete. A specimen of *Trigonocarpus Parkinsoni* in Mr. Kidston's

[1] Scott ('99), pp. 89, 95; Pl. 10, Fig. 5; Pl. 12, Fig. 15.

collection[1] has a micropylar beak almost 3 cm. in length, or quite double that of the body of the seed. As will be pointed out later in this paper it is quite possible that more than one species of *Trigonocarpus* is represented among these casts, and some forms may have had a longer micropylar beak than others.

The series of transverse sections cut at successively higher levels, shown in Pl. XI, Figs. 6–8, and Pl. XII, Figs. 9–11, will serve to illustrate the changes which take place in the micropylar region of this seed. Figs. 6, 8–10 are magnified about six times, Fig. 7 about six and a half, and Fig. 11 about thirty-two times. The sections from which Figs. 9–11 were made are among those which were described by Mr. Wild in the paper previously referred to[2]. The changes which take place in the degree of prominence of the principal and secondary longitudinal ridges of the sclerotesta as they are traced from the body of the seed into the micropylar beak have already been described (p. 107), and are illustrated in Figs. 6–8.

Pl. XI, Fig. 6, is a transverse section across the upper part of the body of the seed, and probably passes through the walls of the pollen-chamber seen at *p*. The central cavity of the seed is beginning to assume a triangular outline, showing that the section is cut quite close to the base of the micropyle. The sclerotesta, *s.t.*, *s'.t'.*, and sarcotesta, *sa.*, are fairly well preserved, and the former shows the usual splitting along the principal ridges. The outer, lacunar part of the sarcotesta is destroyed, with the exception of the limiting layers, *l.*, which can be traced round the greater part of the seed. In some places the limiting layers of the sarcotesta have been so pressed in as to be in contact with the sclerotestal ridges, so that the rest of the sarcotesta in these places has been totally destroyed. There seems no reason to suppose that the transverse section of the complete seed at this level was other than circular.

Pl. XI, Fig. 7, shows a transverse section cut at a somewhat higher level. It evidently traverses the lower thicker part of the micropylar beak. The micropylar canal, *t.m.*, has now acquired its characteristic triangular form, and in one place the sclerotesta, *s.t.*, shows splitting in a position corresponding to one of the principal ridges of the body of the seed. The limiting layers, *l.*, of the sarcotesta can be traced the whole distance round the seed, although in places they have evidently been much disturbed, as is shown by the overlap on the right side. The sarcotesta has the form of two wings, one on either side of the boss formed of the central sclerotic tissue. The total spread of the 'wings' in this slide is about 19 mm., or, allowing for the overlap of about 3·5 mm. on the shorter (right) side, this would make the two sides roughly equal and the total spread of the 'wings' about 21 mm. The width of one side of the triangular micropyle is nearly 2 mm.

[1] Figured in Scott ('05) (1), Fig. 33, p. 146.

[2] Wild ('00).

Pl. XI, Fig. 8, is another transverse section from the same specimen as that shown in Fig. 7, but it is cut at a considerably higher level across the micropylar beak. The sclerotesta, *s.t.*, has become thinner and the ridges are less developed. The triangular form of the micropyle, *t.m.*, is still preserved, although it is no longer equilateral, one side being somewhat shorter than the others. The wing-like form of the sarcotesta is clear, but there has been much loss of tissue and consequent deformation. Allowing, as before, for the overlap on the shorter side, the total spread of the wings would be the same as before, about 21 mm. In some places the sarcotestal tissue, with the exception of the limiting layers, *l.*, has entirely disappeared, and the two surfaces of the wing are pressed together.

Pl. XII, Fig. 9, is another transverse section from the same specimen as Figs. 7 and 8, but is cut at a still higher level. The sclerotesta, *s.t.*, is here very much reduced in quantity, and evidently at a slightly higher level it would disappear altogether. The micropyle, *t.m.*, is still triangular, and the departure from the equilateral form is still more marked than in Fig. 8. The total spread of the 'wings' is almost exactly equal to that of Figs. 7 and 8.

Pl. XII, Fig. 10, is from another slide cut from the same specimen as the others. Sclerotic tissue, *s.t.*, around the micropyle can still be seen, although it is quite small in amount. There is now no sign of a triangular micropyle, but its place is taken by a slit; however, it is quite evident that in this part of the seed, owing to the small amount of sclerotic tissue, a triangular micropyle might readily have been converted into a crevice by crushing from without. Compared with the lower sections, the micropylar boss in this slide is not well defined, while the whole structure varies irregularly in thickness, owing partly to collapse consequent on disappearance of tissue, and partly to the intrusion of Stigmarian rootlets. The best-preserved part of the wing, however, does not look as if much loss of tissue has taken place.

The section shown in Pl. XII, Fig. 11, from another seed, is the highest of our transverse series, and is cut, apparently, near to the extreme tip of the micropyle. This figure is magnified about thirty-two times, or about five times the scale of the other figures in this transverse series. The limiting layers, epidermis, *e.*, and hypodermis, *h.*, are quite well preserved, and the inner tissues are fairly well so. The micropyle appears to be nearly or quite closed, and is surrounded by a little group of brown cells, *b.*, not more than 300 μ in diameter, and that by a light zone, *l.*, the maximum diameter of which is not more than 400 μ, so that the whole is on an extremely small scale. This section apparently shows more than half the circumference of the micropylar tube, and as the maximum distance from the centre of the micropyle to the outside of the testa is only 1·2 mm. there is no question of a wing, unless one supposes that the micropyle is entirely

displaced, which can scarcely be the case as the tissues are fairly well preserved.

It is from the examination of sections such as those shown in Figs. 7–10 (and there are others which exhibit the same features), that the opinion has grown that the long micropylar region of *Trigonocarpus Parkinsoni* was provided with a broad wing continuous with the outer layer of the testa.

We have found it impossible to satisfy ourselves on the question whether the wing is a natural structure or is merely a result of the flattening of the soft tissues of an originally cylindrical sarcotestal investment of the micropyle. In all the sections which we have examined there has certainly been much loss of tissue; sometimes, as in parts of Pl. XI, Fig. 8, where the limiting layers of the sarcotesta are pressed into contact, the whole of the tissue between has disappeared. In the section represented in Pl. XI, Fig. 8, the total spread of the wings, allowing for the overlap, is about 21 mm. Supposing the epidermis filled out to form a circle (i. e. with no wings) the circumference would be about 45–50 mm., which would correspond to a diameter of about 14–16 mm. The seed represented in Pl. XI, Fig. 5, has an average diameter of about 17 mm., but as the outer part of the sarcotesta has been destroyed the diameter in the original condition would doubtless have been somewhat greater than this. Anyhow, the measurements show that if the seed remained circular at the micropylar region there was only slight diminution in size up to the levels represented by Pl. XI, Fig. 8, and Pl. XII, Figs. 9 and 10. On the other hand, if the wing is natural, its spread would have been greater than the diameter of the seed below, which seems very unlikely to have been the case. Taking an intermediate view, that the wings had been somewhat reduced in thickness and exaggerated in spread, we might suppose that the maximum diameter of the seed remained almost constant up to the level of these sections, the micropylar region being flattened instead of cylindrical.

On the circular hypothesis the epidermis, in such a section as Pl. XI, Fig. 8, must have been squashed in upon the micropylar boss, for the diameter here would have been more than double what it is now. Unfortunately, a critical section is not available, as in no slide is the preservation of the tissues sufficiently good to enable us to trace continuity of structure between the sclerotic tissue of the boss and the dark hypodermal layer of the sarcotesta. That there has been loss of tissue is certain, but the structure does not suggest so great a displacement as is necessitated on the circular hypothesis. In other sections, also, the limiting layers of the sarcotesta round the micropylar boss seem to be in nearly their natural position.

On the other hand, the fact that somewhat wing-like appendages may be produced by mere pressure is demonstrated in sections taken across the stalk, or even the body of the seed. Pl. XI, Fig. 5, shows a small 'wing' on one side, while Pl. XI, Fig. 3, from the stalk of the seed, exhibits

a 'wing' comparable in length and form with one from the micropylar region. At the same time the 'wings' in the micropylar region are much more symmetrically developed than elsewhere. We think that, on the whole, the evidence favours the existence of real wings, but the examination of specimens with still better preservation of the outer part of the testa will be necessary before the presence of wings can be positively affirmed.

The Nucellus and Pollen-Chamber.

In all our longitudinal sections of *Trigonocarpus Parkinsoni* the nucellus stands up freely from the base of the seed, with a space between it and the sclerotesta (see Pl. XII, Figs. 12 and 13). The width of this space varies considerably in different specimens, and in some cases it is quite narrow. In a fairly median longitudinal section (941 S.) in which the nucellus does not appear to have suffered much displacement or contraction, the average width of the space is only ·25 mm. In most slides contraction has taken place, and the space is consequently much greater.

On the assumption of an 'inner flesh' (p. 110) this space would have been partly occupied, so that if, as seems probable, the nucellus was free from the integument, the space between must have been a very narrow one.

The nucellus itself is also quite narrow, and in good sections preserves a fairly constant width throughout its extent, from the chalazal attachment below to the base of the pollen-chamber above. The nucellus shown in Pl. XII, Fig. 15, has an average thickness of ·1 mm. In other sections, in which the nucellus is less perfectly preserved, the thickness is commonly not more than ·05 mm., probably owing to the fact that the nucellar epidermis is usually destroyed.

Within the nucellus is a dark-coloured structureless line (Pl. XI, Fig. 5, *m.*), which doubtless represents the membrane of a large, solitary megaspore. The megaspore membrane is always found contracted away from the nucellus, with which it was doubtless originally always in contact. Unfortunately, the prothallus and archegonia are never preserved.

Transverse sections of the body of the seed made at different levels exhibit some characteristic differences in the appearance of the nucellus. In sections across the lower part of the seed the nucellus frequently possesses numerous blunt or pointed outgrowths projecting into the space between it and the testa. These processes are often rather irregular in their distribution and size, and are well shown in some of the original Williamson slides [1]. In sections above the middle of the seed these outgrowths are usually less conspicuous, so that this feature is of some value in determining whether a single transverse section is cut above or below the middle of the seed. With regard to the origin of these processes, we believe that they are caused by the contraction of the tissue of the nucellus which lies between

[1] e.g. No. 1460 W.

them, and that they generally correspond in position with the longitudinal vascular strands to be described later.

The structure of the nucellus, as seen in the best-preserved sections, is shown in Pl. XII, Fig. 15, and Pl. XIV, Figs. 29–31. Externally, there is a single layer of cells forming an epidermal layer, *n.e.*, readily distinguishable from the underlying tissue. These cells are usually filled with dark contents similar in appearance to those of the hypodermal cells of the sarcotesta. In longitudinal sections (Pl. XII, Fig. 15) these cells, *n.e.*, are usually somewhat elongated in the longitudinal direction, and have an average length of about ·08 mm., but vary considerably among themselves in this respect. Fig. 15 is magnified about forty times, and is taken from a portion of the nucellus which is quite low down in the seed, but similar cells can be seen around the nucellus in most parts of this longitudinal section, so that it is clear that they extend from the chalazal attachment upwards. The nucellar epidermis closely resembles that of *Stephanospermum*, described and figured by Prof. Oliver [1].

Pl. XIV, Fig. 29, represents a portion of the nucellus from a section of *Trigonocarpus Parkinsoni* lent by Prof. Oliver, and is magnified about 560 times. The nucellar epidermis is shown at *n.e.*, and, as is commonly the case, the cuticle of the cells, *c.e.*, is separated. In this section the epidermal cells are empty, but usually they are filled with the characteristic dark contents.

Pl. XIV, Figs. 30 and 31, are made from the same section, a good transverse one lent by the Manchester Museum. The magnification is about 160 times. In Fig. 30 the nucellar epidermis, *n.e.*, is poorly preserved, and only the cuticle is clearly seen. In Fig. 31 the cuticle of the nucellar epidermis is shown at *e.*, and the contracted contents of the cells at *c.e.* In many sections the nucellar epidermis is represented only by its cuticle, which persists as a fine line separated by a space from the rest of the nucellus.

Within the epidermis good sections show a tissue consisting of thin-walled flattened cells (Pl. XIV, Fig. 31, *i.*). As shown in Fig. 31, the cells of this intermediate layer are flattened in the radial direction, and as far as can be made out from the longitudinal sections they are elongated vertically. The corresponding cells shown in Pl. XIV, Fig. 30, *i.*, are less flattened, possibly because they are opposite to one of the outwardly directed processes already mentioned.

The innermost layer of the nucellus may be distinguished as the tracheal zone, consisting as it does mainly of delicate tracheides with spiral or scalariform thickenings (Pl. XIV, Fig. 31, *t.*). The vascular bundle which comes in at the chalaza spreads out into what is apparently a continuous mantle of tracheides in the lower part of the seed. In some of the transverse sections across the lower part of the body of the seed the

[1] Oliver ('04) (*1*), Pl. 43, Fig. 21.

tracheides can be traced continuously for about a quarter of the circumference of the nucellus, so that there is no doubt as to their forming a continuous investment to the megaspore in this part of the seed. Higher up in the body of the seed the tracheides appear to range themselves in more or less definite longitudinal bundles, which are connected by transverse anastomoses running in a tangential direction. The structures observed are shown in Pl. XIV, Figs. 30 and 31. Fig. 30 shows a portion of a transverse section of the nucellus, including one of the outwardly directed processes already mentioned. On the inner side of the nucellus a flattened longitudinal nucellar bundle is shown, *n.b.* The tracheides of which the strand is composed are usually much flattened radially, as shown in the figure. In its central part the bundle shown is about three elements thick, but laterally it becomes reduced to but one layer of longitudinally running tracheides.

Pl. XIV, Fig. 31, is drawn from the same transverse section as Fig. 30, and shows at the lower end part of one of the flattened longitudinal bundles, *n.b.*, consisting of fairly thick-walled elements which can be seen to be tracheides in other parts of the section where the nucellus is cut somewhat obliquely. Outside the longitudinally running tracheides—cut transversely in the section—other tracheides, *t.*, are shown running in a horizontal or oblique direction. Laterally, as shown in the upper part of Fig. 31, the longitudinal tracheides disappear, and are replaced by others which run in an approximately horizontal direction, and so form transverse anastomoses between the longitudinal bundles. Similar longitudinal strands are seen in this section opposite to other projections on the outer side of the nucellus.

The nucellar tracheal system has been traced through the whole length of the nucellus, almost to the base of the pollen-chamber. There is no evidence of phloem in connexion with the nucellar tracheides.

A tracheal investment of this kind to the megaspore appears to have been a common feature of many of the old Cycadean seeds of the Palaeozoic era. Prof. Oliver has described a somewhat similar tracheal investment in *Stephanospermum*. In *Stephanospermum akenioides* the structure of the nucellus as a whole is almost identical with that of *Trigonocarpus Parkinsoni*, excepting that, whereas in *Stephanospermum* the tracheal mantle forms a thin but continuous sheath without trace of local segregation into bundles [1], in *Trigonocarpus* there are definite longitudinal bundles.

The question of the relation of the nucellus to the integument, whether coherent with it throughout the greater part of its extent as in the modern Cycadeae, or free from it as in *Stephanospermum* or *Lagenostoma*, is one of importance. From the fact that the nucellar epidermis in *Trigonocarpus* can be traced throughout the whole length of the nucellus from the chalaza upwards, as in *Stephanospermum*, and is not confined to the upper part, as

[1] Oliver ('04) (1), pp. 367, 368.

it probably would be if the nucellus were free only at the tip as in the Cycads, we believe that in *Trigonocarpus* the nucellus was free from the integument throughout its whole length.

The Pollen-Chamber.—Above the prothallial region of the nucellus is seen a relatively small pollen-chamber (Pl. XII, Fig. 13, *p.c.*), a very characteristic feature in these old seeds. This structure in *Trigonocarpus* was indicated in some of the figures of Hooker and Binney [1], and afterwards described and more fully illustrated by Williamson [2]. Curiously enough, the best preservation of the pollen-chamber which we have yet seen is in one of the original Hooker and Binney slides, now preserved in the Binney Collection at Cambridge, and figured in our Pl. XII, Figs. 13 and 14, and Pl. XIV, Fig. 32.

As shown in Pl. XII, Fig. 13, the pollen-chamber forms a wide dome, 2·5–3 mm. in diameter at the base. We have to add the new fact that the pollen-chamber was provided at the top with a narrow channel or beak, *be*, not more than 300 μ in diameter. The presence of a beak is also obscurely indicated in the Williamson section (1478 W.), which is shown in Fig. 114 of his Memoir before referred to. The beak, as shown in the Binney slide, is barely half a millimetre in length, but presumably it extended much farther in the natural condition, and connected the pollen-chamber with the micropyle, or even extended for some distance along the latter.

The pollen-chamber as a whole is smaller than in *Stephanospermum*. In *S. caryoides* the pollen-chamber forms about one-seventh of the height of the whole nucellus, and in *S. akenioides* about one-fourth or one-fifth [3], while in *Trigonocarpus Parkinsoni* the proportion, judging from the Binney section represented in Pl. XII, Fig. 13, and excluding the beak, is about one-tenth. In general structure the pollen-chamber of *Trigonocarpus* appears to have been very similar to that of *Stephanospermum*, but on account of the small number of sections in which the pollen-chamber is shown and the imperfect preservation, it is impossible to describe its structure in much detail. The megaspore wall was doubtless separated from the cavity of the pollen-chamber by a transverse septum continuous with the nucellus. In Pl. XII, Fig. 13, the septum is wholly destroyed, unless the pad of tissue shown at *p.* has been displaced from the floor of the pollen-chamber, as is probably the case. In other sections [4] (not figured) part of the septum remains at the sides.

In *Stephanospermum akenioides* the septum between the megaspore and the pollen-chamber is a vascular sheath consisting largely of tracheides continuous with those of the wall of the nucellus below and around, thus completing the vascular investment of the nucellus [5]. Tracheides are never

[1] Hooker and Binney ('55), Pl. IV, Figs. 7, 8, 12.
[2] Williamson ('77), Pl. XIII and XIV, Figs. 113–15.
[3] Oliver ('04) (1), pp. 363, 374.
[4] e. g. 940 (S); Wild 1939 (S).
[5] Oliver ('04) (1), p. 369.

found beyond the septum, i. e. they never penetrate to the walls or roof of the pollen-chamber itself. In our specimens of *Trigonocarpus* no trace can be seen of tracheal elements either in the septum or in the walls of the pollen-chamber, excepting, perhaps, in the beak to be described later. In the case of the septum this negative evidence does not necessarily imply that tracheides were absent, as the septum in our specimens of *Trigonocarpus* is only poorly preserved, but the absence of tracheides in the pollen-chamber wall in such a slide as that shown in Pl. XII, Fig. 13, when quite well-preserved tracheides are seen lower down in the nucellus in the same section, appears to indicate that such elements were probably really absent. In this respect, then, *Trigonocarpus* probably agrees with *Stephanospermum*.

In all the slides the megaspore membrane (Pl. XI, Fig. 5, *m.*) is contracted away from the nucellus and from the septum above, but doubtless in the natural condition the two tissues were in close contact all round. At the apex, as suggested by Professor Oliver for *Stephanospermum* [1], the megaspore membrane probably bulged the septum upwards, so that the septum and the top of the megaspore encroached somewhat on the pollen-chamber, and may have reduced its size considerably from that which is shown in Pl. XII, Fig. 13, *p.c.* As in *Stephanospermum*, the pollen-chamber was provided laterally with cushions or shoulders of nucellar tissue of considerable thickness, composed of thin-walled elongated cells, the elongation being parallel to the slope of the wall. One of these cushions is shown in Pl. XII, Fig. 13, *c.*, and the other on the opposite side of the pollen-chamber is quite visible in the slide, although not in the photograph. The patch of tissue shown at *p.* in this figure is probably not a portion of one of the cushions, but, as shown by comparison with other slides, part of the floor of the pollen-chamber displaced from its natural position. The tissue of the cushion dies out on the roof of the pollen-chamber.

The wall of the pollen-chamber is covered externally by a layer of epidermal cells apparently similar to, and doubtless continuous with, those of the nucellus below. When traced to the base of the nucellar beak the epidermal cells become radially elongated and palisade-like in form (Pl. XII, Fig. 14, *w.*). A somewhat similar change in the epidermal cells of the pollen-chamber wall has been described by Professor Oliver in *Stephanospermum caryoides*. In this form the flanks of the pollen-chamber are included in a well-marked epidermis of cubical cells which become columnar as the insertion of the beak is approached [2].

In *Trigonocarpus Parkinsoni*, within the epidermal cells of the pollen-chamber beak, there is an inner layer of small prosenchymatous elements, shown in Pl. XII, Fig. 14, *t.*, and Pl. XIV, Fig. 32, *t.* These fibrous elements are not more than about ·01 mm. in width, and when examined under a high power and with good illumination exhibit a very faint 'barring'

[1] Oliver ('04) (1), p. 364.

[2] Oliver ('04) (1), p. 374 and Pl. 44, Fig. 38.

which somewhat resembles tracheal thickenings. The question as to whether these elements are really tracheides or not must be left open for the present, but in view of the absence of tracheides from the wall of the pollen-chamber in both *Trigonocarpus* and *Stephanospermum* we are inclined to think that the elements in question are non-tracheal.

On the whole the pollen-chamber with its beak in *Trigonocarpus Parkinsoni* resembles in a somewhat striking manner the Cordaitean pollen-chamber as shown in Renault's well-known figure [1], while there is also fair agreement with the corresponding structures in a recent Cycadean ovule as in *Stangeria* [2].

IV. Trigonocarpus Oliveri, spec. nov.

This is a new species of *Trigonocarpus* which has kindly been communicated to us for description by Professor F. W. Oliver, F.R.S., after whom it has been named. The locality from which it was obtained—Shore-Littleborough in Lancashire—is a comparatively new one for petrified fossil plants, the colliery having recently been reopened for scientific purposes by Mr. W. H. Sutcliffe, F.G.S. This rich locality has yielded many well-preserved and interesting fossils which are found in nodules from the roof of the workings, as well as in the ordinary nodules or coal-balls lying in the coal-seam immediately below.

Trigonocarpus Oliveri occurs in a roof-nodule, and is at present represented by one specimen only, which has been cut by Mr. Lomax into the four longitudinal sections shown in Pl. XIII, Figs. 19–22. As no transverse sections are available for examination we are unable to fully diagnose the new form, but, as far as can be judged from the longitudinal sections, it is similar in many respects to *Trigonocarpus Parkinsoni*, and we have provisionally placed it in the same genus. At the same time there are differences from the ordinary form which are quite sufficient for its separation as a distinct species.

Trigonocarpus Oliveri is a somewhat smaller seed than *T. Parkinsoni*. None of our sections pass radially through the seed from base to apex, so that the true length of the seed is not shown. The longest section, that shown in Pl. XIII, Fig. 19, has a length of about 1·8 cm., or slightly less than that of *T. Parkinsoni*, specimens of which, excluding the micropylar beak, vary between just over 2 cm. and 2·5 cm. in length. The maximum width of *T. Oliveri* across the body of the seed, measuring to the outside of the sclerotesta, is nearly ·9 cm., or half the length. In *T. Parkinsoni* the corresponding maximum width varies between about 1·3 and 1·6 cm. The new seed is therefore somewhat shorter and narrower, and is also slightly narrower in proportion to its length, than most specimens of *T. Parkinsoni*. The sarcotesta is not preserved, excepting, perhaps, slight traces of it at the

[1] Renault ('81–'85), T. i, Pl. 14, Figs. 5 and 6. [2] e.g. Lang ('00), Pl. XVII, Fig. 15.

base of the seed within the angles of the sclerotesta (Pl. XIII, Fig. 20, *sa.*). The sclerotesta is shown at *s.t.* in the figures and exhibits certain differences from that of the ordinary form. The section figured in Pl. XIII, Fig. 19, is, on the whole, the most median one we have, and shows the characteristic 'coffin-shape' of the sclerotesta and of the cavity within it, which is quite different from the oval form with a rounded base which is seen in all the longitudinal sections of *Trigonocarpus Parkinsoni* (cp. Pl. XII, Fig. 12). This section (Fig. 19) is evidently not quite radial at the base of the seed, as it misses the chalazal attachment of the nucellus *n.* The nucellus shows a ragged appearance due to a number of irregularly disposed processes similar to those described in dealing with the nucellus of *Trigonocarpus Parkinsoni* (p. 119). The base of the sclerotesta is characteristically flat or nearly so, and in this section it has a width, to the outside limit of the sclerotesta, of about ·6 cm.

At the base of the seed the sclerotesta is produced at the angles into two downwardly pointing processes, *p.*, *p.*, which appear to be sections of a circular ridge surrounding the base of the seed. At the apex the section is clearly not radial, as it escapes the micropylar beak altogether. Three processes are seen rising from the top of this section, the interpretation of which is not without difficulty; they probably represent some of the longitudinal ridges of the sclerotesta as they converge to the base of the micropylar beak.

Pl. XIII, Fig. 20, is another obliquely longitudinal section of the same seed. The basal part of the seed is evidently cut nearly radially, as the chalazal attachment of the nucellus, *n.*, is shown. In the upper part of the seed the pollen-chamber, *p.c.*, is apparently cut through. The base of this section shows the downwardly directed processes of the sclerotesta, *p.p.*, better developed than in Fig. 19, and we think that these two sections make it practically certain that there was a circular ridge around the base of this seed, a feature which constitutes one of the most distinctive characters of the species. In Fig. 20 these processes (*p.p.*) evidently extended farther downward than is shown in the specimen; only the proximal part of the circular ridge is preserved.

The upper part of this section (Pl. XIII, Fig. 20) is not cut in the median plane. The three ridges shown, *r.*, *r.*, *r.*, give rise to an appearance as though the apex of the seed was provided with a circular ridge similar to the crown of a *Stephanospermum.* This appearance, however, is probably deceptive, and is due to the obliquity of the section, which thus passes through three of the ordinary longitudinal ridges of the sclerotesta. Three similarly placed ridges are sometimes shown in *Trigonocarpus Parkinsoni* when the seed has been cut obliquely, and the two outermost of these have frequently been described as caused by the presence of an annular ridge. Truly radial sections of *T. Parkinsoni* passing through the micropyle show

no trace of an annular ridge. That the three processes shown in Pl. XIII, Fig. 20, *r.*, *r.*, *r.*, are only longitudinal ridges is also made clear by their absence in the more radial section of the upper part of the same seed shown in Pl. XIII, Fig. 19.

Pl. XIII, Fig. 21, is a vertical tangential section, and shows the cavity of the seed, *c.*, and the surrounding sclerotesta, *s.t.* The sclerotesta is much thicker on one side than on the other, probably because the section passes through one of the longitudinal ridges on that side. On the thicker side a nearly detached portion of the sclerotesta is shown, probably corresponding to another longitudinal ridge.

Pl. XIII, Fig. 22, is still more tangential, as it shows little more than the sclerotesta, *s.t.*, with a trace of the cavity of the seed within. The sharply pointed upper and lower ends of this section probably indicate that the section passes through one of the longitudinal ridges, and that these had very acute edges. In *Trigonocarpus Parkinsoni* the ridges were more blunt or round, and none of the numerous sections which we have examined of this form are at all like the section shown in Pl. XIII, Fig. 22. We do not think that the form represented in this figure is a possible one for *Trigonocarpus Parkinsoni.*

The innermost cells of the sclerotesta, especially in the section shown in Fig. 22, have dark contents and are quite different from the rest of the cells of this layer. They are continued as fine lines from the centre of the section (Fig. 22) through the middle of the longitudinal ridge shown above and below. The splitting of the testa along the ridges, which can be seen in Figs. 20 and 22, appears to proceed along these cells, which seem to be in a state of disorganization. In this form the splitting of the testa along the ridges would thus appear to have been a natural process correlated with the presence of special cells occupying the central parts of the sclerotestal ridges.

The presence of a circular ridge surrounding the base of the seed, and enclosing, presumably, the stalk by which it was attached to the parent plant, is a character which *Trigonocarpus Oliveri* possesses in common with *Polylophospermum stephanense*, a French seed which has been recently re-described by Professor Oliver [1]. Indeed longitudinal sections of the base of this seed compare closely with similar sections of *Trigonocarpus Oliveri.*

Polylophospermum stephanense is a long prismatic seed, hexagonal in transverse section and prominently ribbed along the salient angles, while both at the apex and base there are cup-shaped incurved outgrowths of the testa which produce two false chambers at the ends of the seed. One of these chambers encloses the micropylar beak, and the other the stalk of the seed. The length of this seed is about 1·5 cm., or somewhat less than that of *Trigonocarpus Oliveri.*

[1] Oliver ('04) (2).

Trigonocarpus Oliveri differs essentially from *Polylophospermum stephanense* in its general shape and in the absence of the cup-shaped outgrowth of the testa around the micropyle. Its general shape is much less narrow and cylindrical, the proportion of length to breadth being about 2 : 1, while in *Polylophospermum* it is about 3 : 1. The shape in transverse section cannot be determined from our sections.

On the whole, judging from the characters which are available in a series of longitudinal sections of a single seed, we think that this seed more closely resembles *Trigonocarpus*, and we have therefore provisionally placed it in this genus.

The following is a brief diagnosis of the distinctive characters of the new seed :—

Trigonocarpus Oliveri, sp. nova.

Horizon. Lower Coal Measures. Shore-Littleborough, Lancashire.

Size. Length nearly 2 cm. ; maximum transverse diameter about ·9 cm.

Shape. Characteristically 'coffin-shaped' in vertical sections. Base flattened. Sclerotesta produced around the base of the seed in the form of a circular ridge enclosing the stalk of the seed. Longitudinal ridges of the sclerotesta acute-angled, not rounded as in *Trigonocarpus Parkinsoni.*

V. The Casts and Impressions.

It is not intended in this paper to enter into a full discussion of the specimens of *Trigonocarpus* which do not exhibit internal structure—the internal casts of the cavity of the seed and the more or less flattened specimens of the whole seed which have usually been described under the name of *Carpolithes alata* (Lindley and Hutton). At the same time, a correlation of the features shown in those specimens with those of the petrified seeds showing internal structure is of some interest and importance, and we propose to devote some attention to this matter in the present paper, especially with reference to the three good specimens of *Carpolithes alata* represented in Pl. XII, Figs. 16 and 17, and Pl. XIII, Fig. 18.

Taking first the ordinary internal casts, some account has already been given of these nut-like 'seeds' in the present paper (pp. 90, 94), and a few additional details may be given here. The common type of these internal casts—those described under the names of *Trigonocarpus Parkinsoni* and *T. olivaeformis*—although they vary somewhat among themselves in size and shape, still agree closely with the petrified specimens showing internal structure, so that it is highly probable that they represent the same form. The probable identity of the casts described as *T. Parkinsoni* and *T. olivaeformis* has already been pointed out (p. 93).

Many of the internal casts show a triangular fracture at the narrower (upper) end, which doubtless represents the place where the cast of the

triangular micropylar tube had broken off. Many of the specimens also show a circular or oval scar at the lower or more rounded end; this scar probably indicates the edge of the tracheal disk from which the free nucellus sprang (see Pl. XII, Fig. 12, *p*.). The internal casts show, as a rule, three well-marked longitudinal ridges, which doubtless represent the three longitudinal furrows on the inner side of the sclerotesta of *Trigonocarpus Parkinsoni*. We have described (p. 108) how these furrows become less deep or pronounced towards the base of the body of the seed in petrified specimens; it is interesting to find that the internal casts show the three ridges dying out towards the bottom, exactly as shown in the sections. None of the sections of *Trigonocarpus Parkinsoni* which we have examined would yield internal casts with more than three longitudinal ridges, so that the casts described as *Trigonocarpus Noeggerathi* (Stern.), which are in size and shape comparable with ours, but are provided with six longitudinal ridges instead of three, must have belonged to a different but probably closely related plant.

We pass now to the impressions shown in Pl. XII, Figs. 16 and 17, and Pl. XIII, Fig. 18, which are similar to those described by Lindley and Hutton as *Carpolithes alata*, and identified by Mr. Kidston with *Trigonocarpus Parkinsoni*[1]. Whether the three specimens figured here and others figured elsewhere all belong to the same species may perhaps be left for the present an open question, but that they are specimens of *Trigonocarpus* seems certain, although the correlation of the features presented by these impressions with the parts of the structural specimens presents considerable difficulty.

The specimens shown in Pl. XII, Figs. 16 and 17, now belong to the Copenhagen Museum, but were originally contained in the Hutton Collection at Newcastle. The specimens were obtained for us through the good offices of Mr. E. A. Newell Arber, F.G.S. The photographs are about twice the natural size, so that the total length of the specimens as preserved is about 4·5–5 cm. Of the total length, the rounded body of the seed forms about four-ninths in the specimen shown in Fig. 16, and about half of that shown in Fig. 17. In a figured specimen of *Trigonocarpus Parkinsoni* belonging to Mr. Kidston[2] the body of the seed forms only one-third of the total length. The latter specimen may represent a different species from ours, unless in our specimens the micropylar beak is incompletely preserved. Of the three examples figured in this paper (Pl. XII, Figs. 16 and 17, and Pl. XIII, Fig. 18), one, Fig. 17, has every appearance of being complete at the upper end, but in the other specimens (Figs. 16 and 18) the micropylar beak quite probably extended farther than shown, especially so as we know that the upper portion of the beak consisted almost wholly of thin-walled, easily destroyed tissue.

The specimen shown in Pl. XII, Fig. 16, is probably to be interpreted

[1] Kidston ('86), pp. 218, 219.

[2] Figured in Scott ('05) (1), Fig. 33, p. 146.

in the following way. The outer surface of the sclerotesta surrounding the micropylar canal is shown at *s.t.b.*, the reference line passing to one of the longitudinal ridges of the micropylar beak (compare the transverse section across the lower part of the micropylar beak shown in Pl. XI, Fig. 7). The beak shown in Fig. 16 gradually tapers upwards, so that while the width at the base is about 6 mm., at the upper end it is less than 3 mm. These dimensions agree approximately with those of the sclerotesta shown in Pl. XI, Figs. 7 and 8, from which it appears that these two sections were cut at a considerable distance from one another, perhaps nearly equal to the length of the micropylar beak shown in Fig. 16. The upper end of the micropylar beak of Fig. 16 has an unfinished appearance, probably because the distal portion, consisting of thinner-walled tissue, is lost. That the outer surface of the sclerotesta of the beak, and not that of an internal cast of the micropylar canal, is shown in Fig. 16, is made probable, then, by its size agreeing with that of the petrified specimens. The width of the base of the beak compared with that of the body of the seed also confirms this explanation. A typical transverse section across the body of the seed, such as that shown in Pl. XI, Fig. 5, has a diameter (measuring to the outside of the sclerotesta) of about 1·3 cm., while the diameter at the base of the beak is almost exactly half this, and the same proportion holds good in the specimen represented in Pl. XII, Fig. 16. On one side of the micropylar beak is seen a flat area (Pl. XII, Fig. 16, *sa.*), which we interpret as the impression of the sarcotestal covering. The appearance presented might very well be due to a wing of soft tissue more or less 'doubled' under. This 'wing' cannot be traced with certainty below the beak portion of the seed.

The body of this seed (Fig. 16) has such a form and dimensions as to quite well agree with the petrified specimens of *Trigonocarpus Parkinsoni*. The body of the seed is split in such a way as to provide us with a tangential section showing the sclerotesta, *s.t.*, preserved in coaly material which is continuous with the similar substance forming the micropylar beak, *s.t.b.*, and the material, *c.*, filling up the central cavity of the seed.

Pl. XII, Fig. 17, shows another seed impression which has a remarkable appearance of being complete, especially at the upper end, so that the whole seed has a symmetrical, elongated, oval outline. The body of this seed is about equal in width to that of the specimen shown in Fig. 16, but its length is about ·5 cm. greater. The micropylar beak, *m.b.*, can be seen extending from the top of the body of the seed for a distance of 2·25 cm., preserving an almost equal size the whole distance. The lower (darker) portion of the beak, 1 cm. in length, is preserved in relief, and has a triangular form, with an edge or longitudinal ridge to which the reference line, *m.b.*, passes, and possibly another on the left of this. The upper (lighter) part of

the beak is represented by a furrow with a median groove, because the actual micropylar beak has been broken off and only its impression remains.

The body of the seed shown in Fig. 17 presents much the same appearance as that of Fig. 16; what appears to be the sclerotesta is shown at *s.t.*, while *c* is perhaps the surface of the coaly shale which forms a cast of the interior cavity. A fact which is difficult of explanation, on the supposition that *c* in Figs. 16 and 17 represents the surface of an internal cast of the cavity of the seed, is the complete absence of any sign of the longitudinal ridges which are so characteristic a feature of the ordinary sandstone internal casts of *Trigonocarpus*. Mr. E. A. Newell Arber informs us that he has seen hundreds of such smooth specimens. The absence of longitudinal ridges would be equally difficult to explain if we assume that the smooth surface *c* represents the surface of the whole seed squashed flat, for we should expect the sclerotestal ridges of the body of the seed to be shown.

The most interesting feature of the specimen shown in Pl. XII, Fig. 17, is the impression of the sarcotesta, *sa.*, which surrounds the whole seed and gives to it a regular, elongated, oval form. The whole seed may have been symmetrically flattened out into the form shown, or, if a real wing was present, it is probably seen in this specimen. On the whole, we think that the specimens shown in Figs. 16 and 17 favour the presence of a real wing, although the evidence from the impressions, as well as from the study of the petrified specimens, is not conclusive.

The third of our figured specimens of *Carpolithes alata* (*Trigonocarpus Parkinsoni*?) is shown in Pl. XIII, Fig. 18, the magnification being equal to that of the others. The specimen comes from Jarrow, and belongs to the Hutton Collection in the Newcastle Museum. The total length of the seed as shown is about 4 cm., the body of the seed being 1·8 cm. and the micropylar beak 2·2 cm. in length, while the maximum width of the body is 1·5 cm. The size is therefore approximately equal to that of the other two impressions and to the ordinary petrified specimens. The micropylar beak, *m.b.*, has a triangular form, and tapers rather rapidly above. The body of the seed and the micropylar beak are apparently represented as internal casts in the coaly shale. One of the internal grooves of the sclerotesta is represented by the conspicuous ridge, *r.*, which is seen passing across the body of the seed, and continuing as the prominent edge of the triangular micropyle and traceable almost to the summit of the latter. That the surface of the body of the seed shown is really that of an internal cast, and not the outer surface of the sclerotesta, is clear from the fact that but one longitudinal ridge can be seen either here or along the micropylar tube. Preservation of the outer surface of the sclerotesta sufficiently good to show one well-marked ridge would be practically certain to show others also. Moreover, the sclerotesta itself is seen in this specimen as a layer of coaly material, *s.t.*, which encloses the internal cast in places, although the greater part of it has been broken away.

One difficulty in the interpretation of the specimen shown in Pl. XIII, Fig. 18, is that the longitudinal ridge apparently extends to the bottom of the body of the seed, while, as we have before mentioned in this paper, the ordinary internal casts usually show the ridges disappearing towards the base of the seed. Examination of a number of specimens of *Carpolithes alata* and of the ordinary internal casts at the Natural History Museum makes it highly probable that in Fig. 18 the real base of the seed is not seen. The ridge shown is of exactly the same length as one of the ridges of many of the internal casts, and the real base of the seed was in all probability unribbed. We feel little doubt as to the specimen shown in Fig. 18 being really mainly preserved as an internal cast, with a thin coaly layer representing the sclerotesta preserved in places.

The true interpretation of impressions such as those shown in Figs. 16, 17, 18, presents considerable difficulty, and becomes possible only after the internal structure of the petrified specimens has been worked out in detail.

References.

Brongniart ('28): A. Brongniart. Prodrome d'une histoire des végétaux fossiles. Paris.

——— ('49): Tableau des genres de végétaux fossiles (Dict. univ. d'hist. nat., vol. xiii). Paris.

——— ('74): Ann. des Sci. Nat., Bot., tome xx, pp. 234-65.

——— ('81): Recherches sur les graines fossiles silicifiées. Paris.

Hooker and Binney ('55): J. H. Hooker and E. W. Binney, On the structure of certain Limestone Nodules enclosed in seams of Bituminous Coal, with a description of some Trigonocarpons contained in them. Phil. Trans., vol. cxlv.

Kidston ('86): R. Kidston, Catalogue of the Palaeozoic plants in the Department of Geology and Palaeontology. British Museum, London.

Lang ('00): Lang, Development of Cycadean Sporangia, II. Ann. Bot., vol. xiv.

Lindley and Hutton ('31-'37): J. Lindley and W. Hutton, The Fossil Flora of Great Britain. 3 vols. London.

Oliver ('04) (1): F. W. Oliver, On the Structure and Affinities of *Stephanospermum*, Brongn., a genus of fossil Gymnosperm seeds. Trans. Linn. Soc. Lond. Botany, vol. vi.

——— ('04) (2): Notes on *Trigonocarpus*, Brongn., and *Polylophospermum*, Brongn., two genera of Palaeozoic Seeds. New Phytologist, vol. iii.

Parkinson ('11): J. Parkinson, Organic Remains of a former World. London.

Renault ('81-'85): B. Renault, Cours de Botanique Fossile.

Scott and Maslen ('06): D. H. Scott and A. J. Maslen, Note on the Structure of *Trigonocarpon olivaeforme*. Ann. Bot., vol. xx.

Scott ('99): D. H. Scott, On the Structure and Affinities of Fossil Plants from the Palaeozoic Rocks. III. On *Medullosa anglica*, a new Representative of the Cycadofilices. Phil. Trans. Roy. Soc. Series B, vol. cxci.

——— ('05) (1): What were the Carboniferous Ferns? Jour. Roy. Micro. Soc.

——— ('05) (2): The Early History of Seed-bearing Plants, as recorded in the Carboniferous Flora. Wilde Lecture. Memoirs of the Manchester Literary and Philosophical Society, vol. xlix, No. 12.

STOPES ('04): Miss M. C. Stopes, Beiträge zur Kenntnis d. Fortpflanzungsorgane der Cycadeen. Flora, Bd. IV.

— — ('05): On the Double Nature of the Cycadean Integument. Ann. Bot., vol. xix.

WILD ('00): G. Wild, On new and interesting features in *Trigonocarpon olivaeforme*. Trans. Manchester Geol. Soc., Part xv, vol. xxvi.

WILLIAMSON ('77): W. C. Williamson, On the Organization of the Fossil Plants of the Coal Measures. Part viii. Phil. Trans. Roy. Soc., vol. clxvii.

EXPLANATION OF PLATES XI–XIV.

Illustrating Messrs. Scott and Maslen's paper on *Trigonocarpus*.

PLATE XI.

Fig. 1. First of a series of transverse sections across a seed of *Trigonocarpus Parkinsoni*. This section is the lowest of the series and is cut across the base of the seed some considerable distance below the chalaza. Slide 627 (S)[1]. Magnification between 6 and 7 times.

b. triangular space containing main supply bundle of the seed and surrounded by a zone of dark-coloured sclerotic tissue continuous with the sclerotesta; *l.* limiting layers of sarcotesta.

Fig. 2. The central portion of the same slide as shown in Fig. 1 more highly magnified. Magnification 100.

s.t. sclerotic tissue continuous with sclerotesta; *b.* main supply bundle of seed; *s.* triangular space enclosing bundle.

Fig. 3. Transverse section of same specimen at a higher level across the base of the seed. Inner part of sarcotesta preserved with about six sarcotestal bundles. Slide 626 (S). Magnification 6.

b. main supply bundle of seed; *s.t.* sclerotestal core surrounding bundle; *s.b.* six sarcotestal bundles embedded in compact sarcotesta; *l.* limiting layers of the sarcotesta.

Fig. 4. Central part of same more highly magnified. *s.t.* part of core of sclerotesta; *b.* main supply bundle of seed; *a.* cells surrounding bundle.

Fig. 5. Transverse section across the central part of the body of the same seed as shown in Figs. 1–4, showing general form and three principal and nine secondary sclerotestal ridges. Slide 325 (S). Magnification 6.

m. megaspore membrane (contracted); *n.* nucellus; *s'.t'.* one of the principal ridges of the sclerotesta; *s.t.* one of the secondary ridges of the sclerotesta; *sa.* sarcotesta; *l.* limiting layers of sarcotesta; *s.b.* position of one of the sarcotestal bundles (shown more magnified in Pl. XIV, Fig. 28).

Fig. 6. Transverse section across the upper part of the body of the seed probably passing across the pollen-chamber. The central cavity is beginning to assume a triangular form and the sclerotesta shows the differentiation into two zones. Slide 1470 (W). Magnification 6.

p. wall of pollen-chamber; *s.t.* inner zone of sclerotesta; *s'.t'.* outer zone of sclerotesta; *sa.* sarcotesta; *l.* limiting layers.

Fig. 7. Transverse section near base of micropyle showing triangular form of micropyle and 'wing' of sarcotesta. Slide Q. 585 (M). Magnification 6¼.

t.m. triangular micropyle; *s.t.* sclerotesta of micropylar beak; *sa.* sarcotesta; *l.* limiting layers of sarcotesta.

Fig. 8. Transverse section across micropylar beak at a higher level than Fig. 7. From same specimen as Fig. 7. Slide 487 (S). Magnification 6.

t.m. triangular micropyle; *s.t.* sclerotesta of micropylar beak; *sa.* sarcotesta; *l.* limiting layers of sarcotesta.

[1] [The reference letters after the number of the slide refer to the collections to which the slides belong. O = in the collection of Prof. Oliver, F.R.S. M = in the Manchester Museum. B = the Binney Collection at Cambridge University. S = Dr. Scott's Collection. W = Williamson Collection at the Natural History Museum.]

PLATE XII.

Fig. 9. Transverse section across micropylar beak at a still higher level. Slide 1943 Wild (S). Magnification 6.

t.m. triangular micropyle; *s.t.* sclerotestal tissue surrounding micropyle; *l.* limiting layers of sarcotesta.

Fig. 10. Transverse section across beak at a still higher level. Same specimen as Fig. 9 Slide 1942 Wild (S). Magnification 6.

s.t. small amount of sclerotic tissue surrounding micropyle which was apparently slit-like in form; *l.* limiting layers of sarcotesta.

Fig. 11. Transverse section of extreme tip of micropylar beak. Opening of micropyle apparently blocked. Slide 1952 Wild (S). Magnification 32.

b. dark cells surrounding micropyle; *l.* light-coloured zone surrounding micropyle; *h.* hypodermis of sarcotesta; *e.* epidermis of sarcotesta.

Fig. 12. Longitudinal section of the lower part of a seed of *Trigonocarpus Parkinsoni*. The section is not strictly radial and therefore does not pass through the chalazal bundle. The attachment of the apparently free nucellus is shown. Slide R. 763 (M). Magnification 7.

n. nucellus; *p.* chalazal attachment of nucellus; *s.b.*, *s.b.* position of sarcotestal bundles one of which is shown more highly magnified in Pl. XIV, Fig. 25; *s.t.* sclerotestal tissue at base of stalk of seed.

Fig. 13. Longitudinal section of upper part of seed showing pollen-chamber and beak. Slide 224 (B). Magnification 6.

p.c. pollen-chamber occupying apex of nucellus; *c.* lateral cushion at side of pollen-chamber; *p.* pad of tissue probably displaced from the floor of pollen-chamber; *be.* beak of pollen-chamber; *s.t.* sclerotesta at base of micropylar beak.

Fig. 14. The pollen-chamber beak of Fig. 13 more highly magnified. Magnification 107.

w. wall of beak consisting of palisade-like cells (see Pl. XIV, Fig. 32); *t.* possible tracheides forming inner portion of wall of beak.

Fig. 15. Portion of the nucellus showing the covering of epidermal cells. From the lower part of a seed. Slide 222 (B). Magnification 40.

n.e. nucellar epidermis; *i.n.* inner tissue of nucellus.

Fig. 16. *Carpolithes alata* (*Trigonocarpus Parkinsoni* ?). From a specimen in the Copenhagen Museum originally contained in the Hutton Collection at Newcastle. Magnification 2.

s.t.b. outer surface of sclerotesta bounding the micropylar beak; *sa.* impression of sarcotesta; *s.t.* sclerotesta of body of seed; *c.* substance filling cavity of seed.

Fig. 17. Another specimen from the same collection. Magnification 2.

m.b. micropylar beak; *sa.* impression of sarcotesta; *s.t.* sclerotesta of body of seed; *c.* substance occupying cavity of seed.

PLATE XIII.

Fig. 18. *Carpolithes alata* (*Trigonocarpus Parkinsoni* ?). Specimen from the Hutton Collection in the Newcastle Museum. Showing internal cast of body of seed with sclerotestal covering and internal cast of micropylar canal.

r. ridge of cast; *s.t.* sclerotestal covering; *m.b.* micropylar beak (internal cast).

Fig. 19. *Trigonocarpus Oliveri*. Longitudinal section of seed showing characteristic 'coffin shape' of the sclerotesta. Slide S. 28, *b.* (O). Magnification 6½. Loc. Shore-Littleborough (roof-nodule).

n. nucellus; *s.t.* sclerotesta; *p.*, *p.* processes of sclerotesta representing annular ridge at base of seed.

Fig. 20. Another vertical section of the same specimen, more median below but less median above. Slide S. 28, *c.* (O). Magnification 6½.

n. nucellus; *p.c.* pollen-chamber; *s.t.* sclerotesta; *sa.* sarcotesta (?) preserved in the angles of the sclerotesta; *p.*, *p.* processes due to annular ridge at base of seed; *r.*, *r.*, *r.* three of the longitudinal ridges of the seed which are cut through on account of the obliquity of the section.

Fig. 21. More tangential section of the same seed passing through a longitudinal ridge of the sclerotesta on one side. Slide S. 28, *d.* (O). Magnification 6½.

s.t. sclerotesta; *c.* cavity of seed.

Fig. 22. Still more tangential section of the same seed showing the sharply angular form of the ridges. Slide S. 28 *a.* (O). Magnification 6½.

s.t. sclerotesta; *c.* trace of cavity of seed.

PLATE XIV.

Trigonocarpus Parkinsoni.

Fig. 23. The limiting layers of the sarcotesta. Slide R. 763 (M). Magnification about 40.

e. epidermis of small cells; *h.* hypodermis of larger cells with dark contents; *sa.* ordinary cells of the sarcotesta.

Fig. 24. Lacunar tissue of the outer part of the sarcotesta. Slide 1459 (W). Magnification about 45.

Fig. 25. Sarcotestal bundles at base of seed. Same slide as Pl. XII, Fig. 12. Slide R. 763 (M). Magnification about 80.

sa. cells of sarcotesta; *b.*, *b.*, *b.* vascular bundles; *s.t.* short tracheides (transfusion elements) in delicate tissue between the bundles; *a.* other short tracheides among cells of sarcotesta; *h.* hypodermal cells of sarcotesta (displaced).

Fig. 26. Types of tracheides from the sarcotestal bundles shown in Fig. 25. Slide R. 763 (M).

A. Commonest type of tracheide with very fine spiral or scalariform markings. Magnification about 280.

B. One of the small elements (protoxylem) from the central part of a bundle. Magnification about 460.

C. Tracheide with two series of crossing spiral bands. Magnification about 280.

D. Oblique view of a scalariform tracheide.

Fig. 27. Two short tracheides (transfusion elements) from the same slide. Slide R. 763 (M). Magnification 560.

Fig. 28. A sarcotestal bundle. Apparently mesarch. The position of this bundle is shown in Pl. XI, Fig. 5, *s.b.* Slide 325 (S). Magnification 200.

sa. cells of sarcotesta; *d.t.* decomposed tissue. The arrow points towards the outside of the seed.

Fig. 29. Portion of the nucellus showing the nucellar epidermis. Slide 4 (O). Magnification 560.

n.e. nucellar epidermis; *c.e.* separated cuticle of epidermal cells; *i.e.* inner tissue of nucellus.

Fig. 30. Part of a transverse section of the nucellus showing a nucellar bundle. Slide R. 762 (M). Magnification 170.

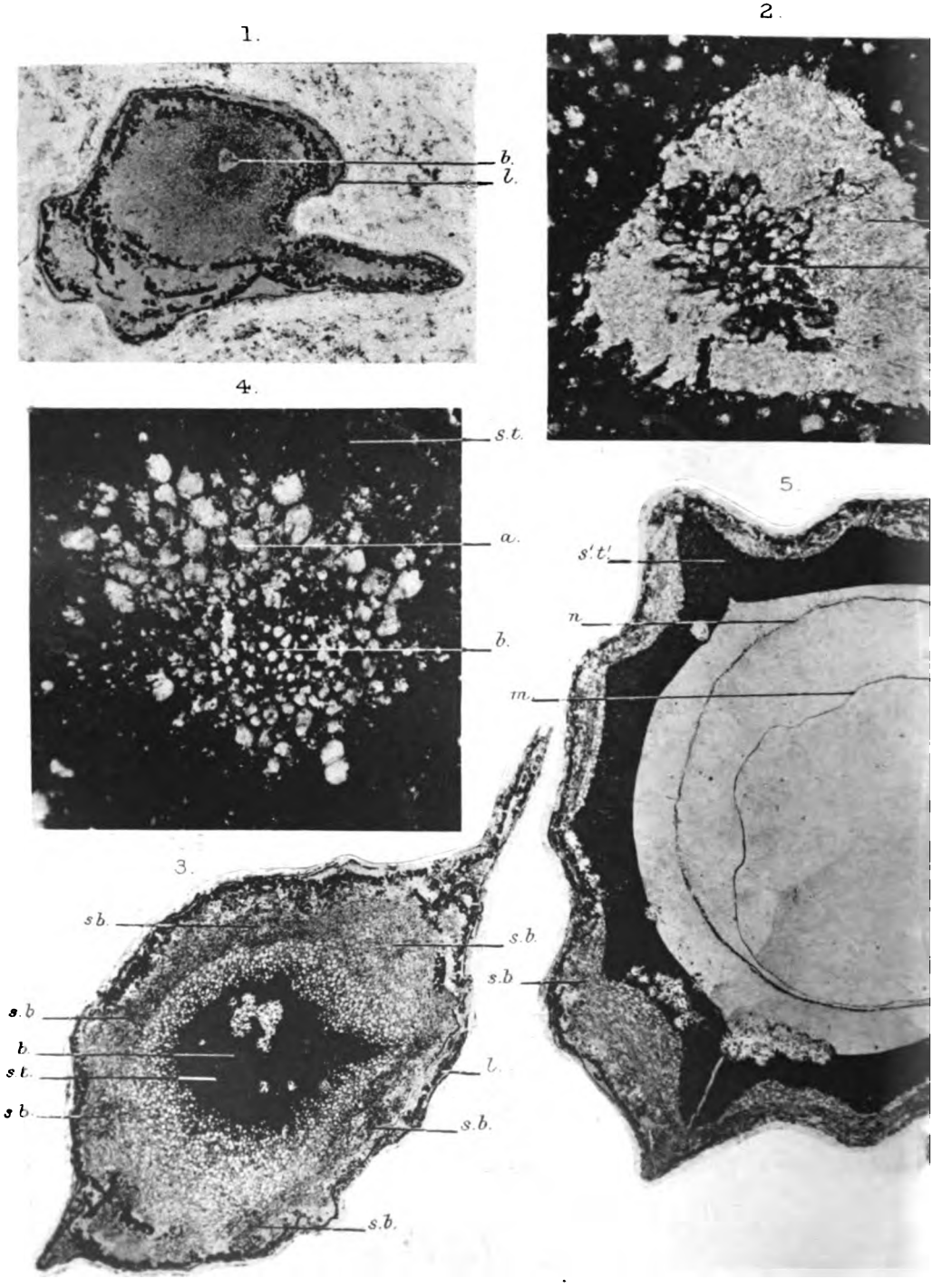
n.b. flattened nucellar bundle; *i.* intermediate layers of nucellus; *n.e.* remains of nucellar epidermis.

Fig. 31. Another part of the same transverse section. Slide R. 762 (M). Magnification 160.

n.b. part of flattened nucellar bundle; *t.* tracheide in longitudinal section; *i.* intermediate layers of nucellus consisting of flattened cells; *c.c.* contracted contents of epidermal cells; *e.* cuticle of epidermal cells.

Fig. 32. A part of the pollen-chamber beak shown in Pl. XII, Fig. 14. Slide 224 (B). Magnification 400.

w. wall of beak consisting of a palisade-like tissue; *t.* possible tracheides.



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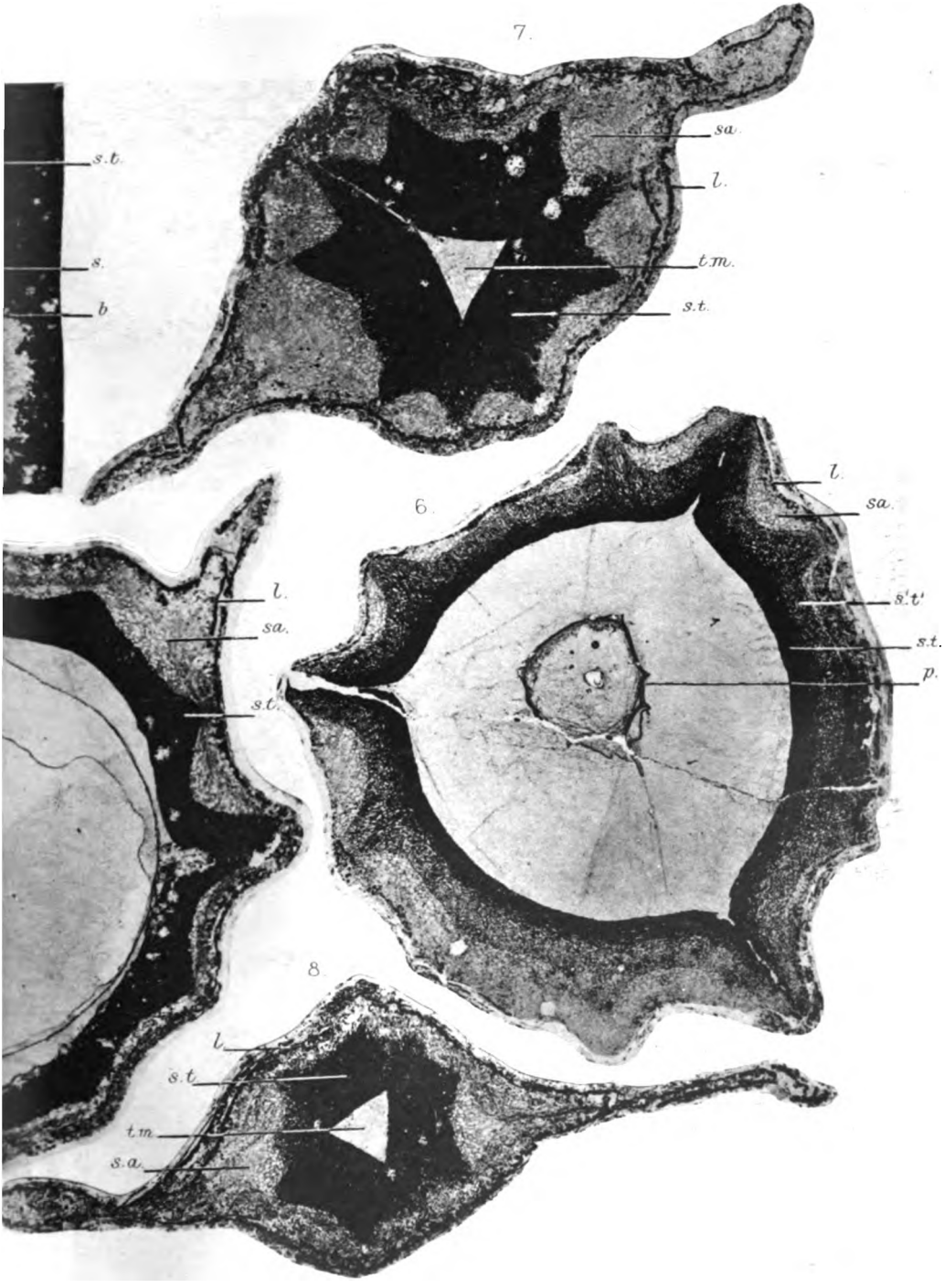

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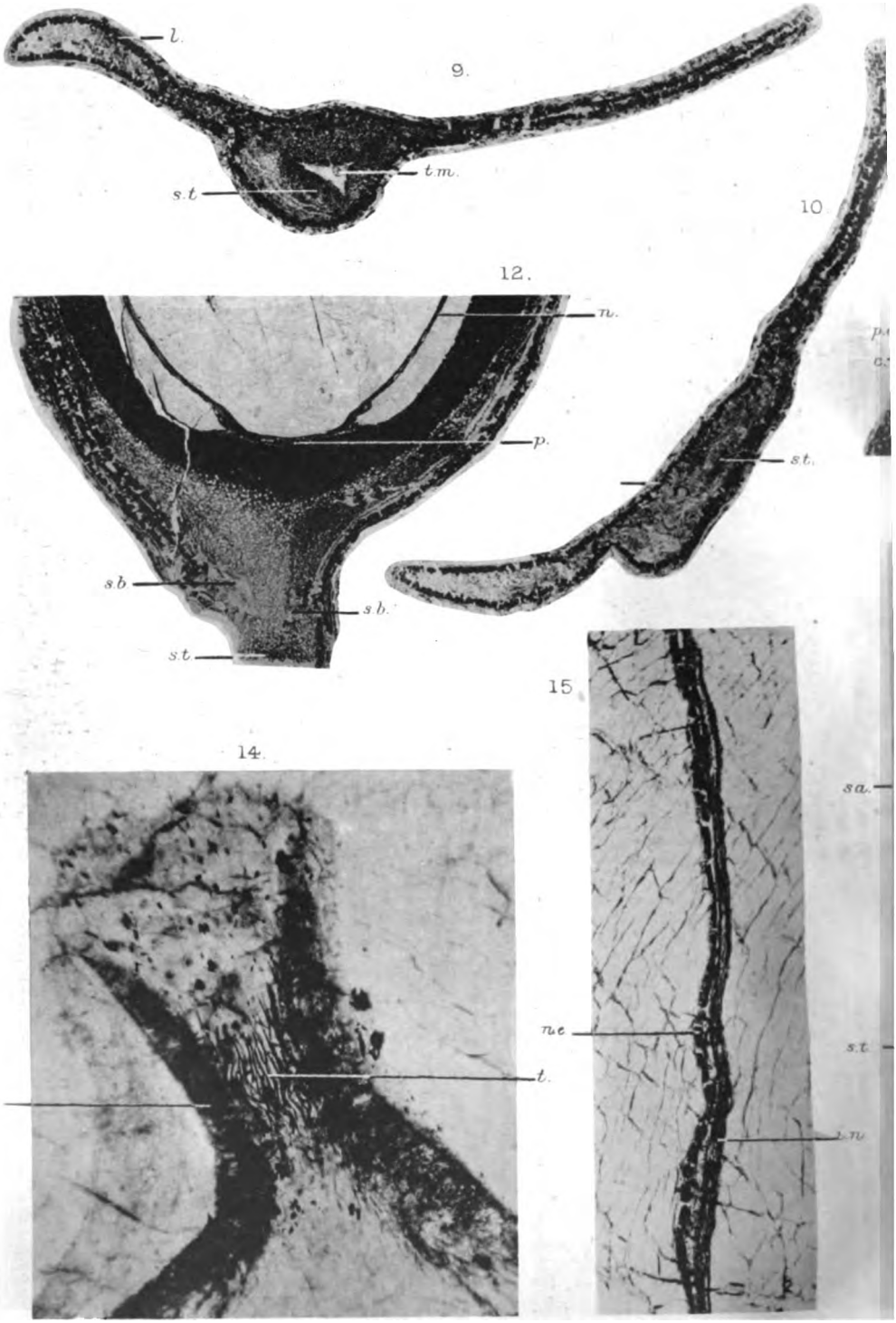

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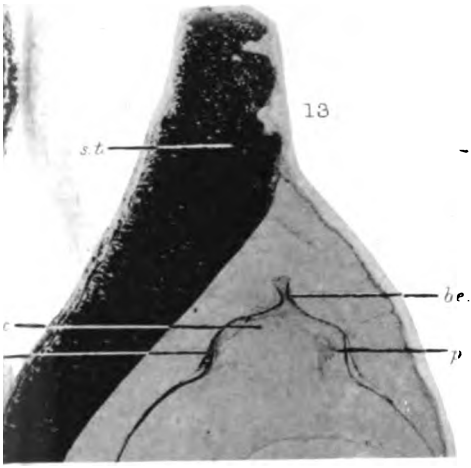

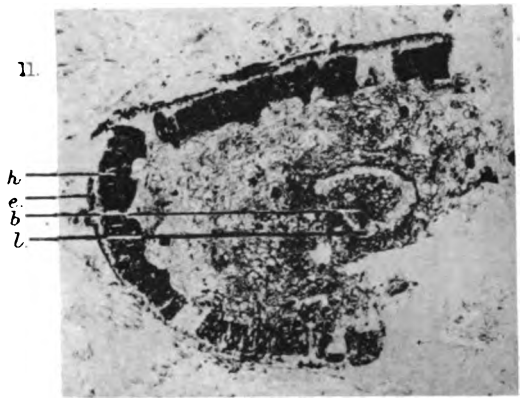

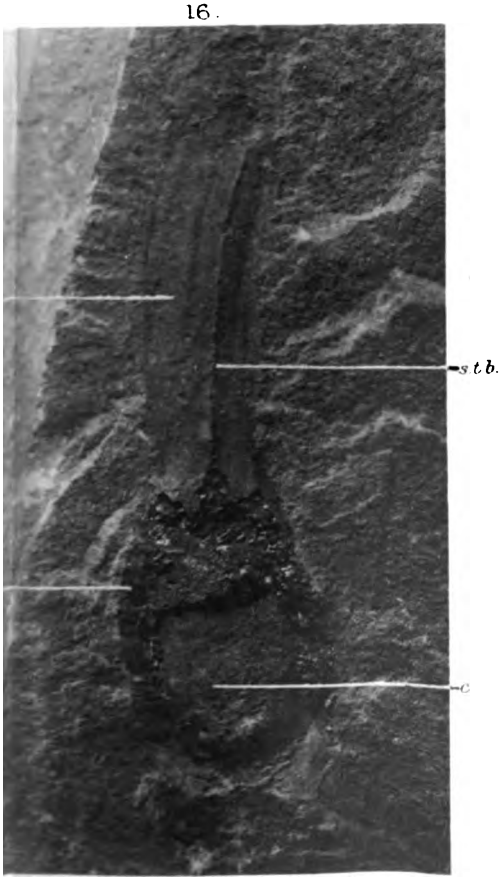

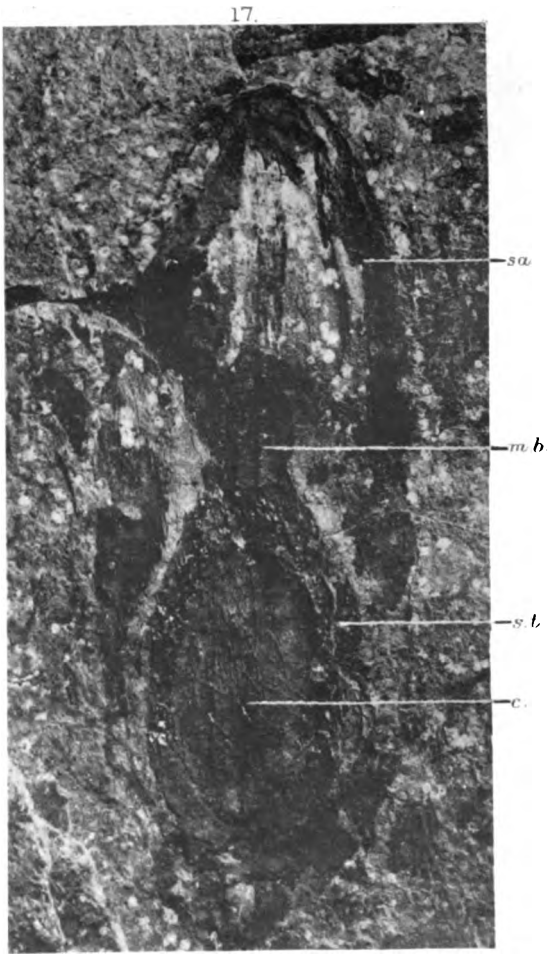

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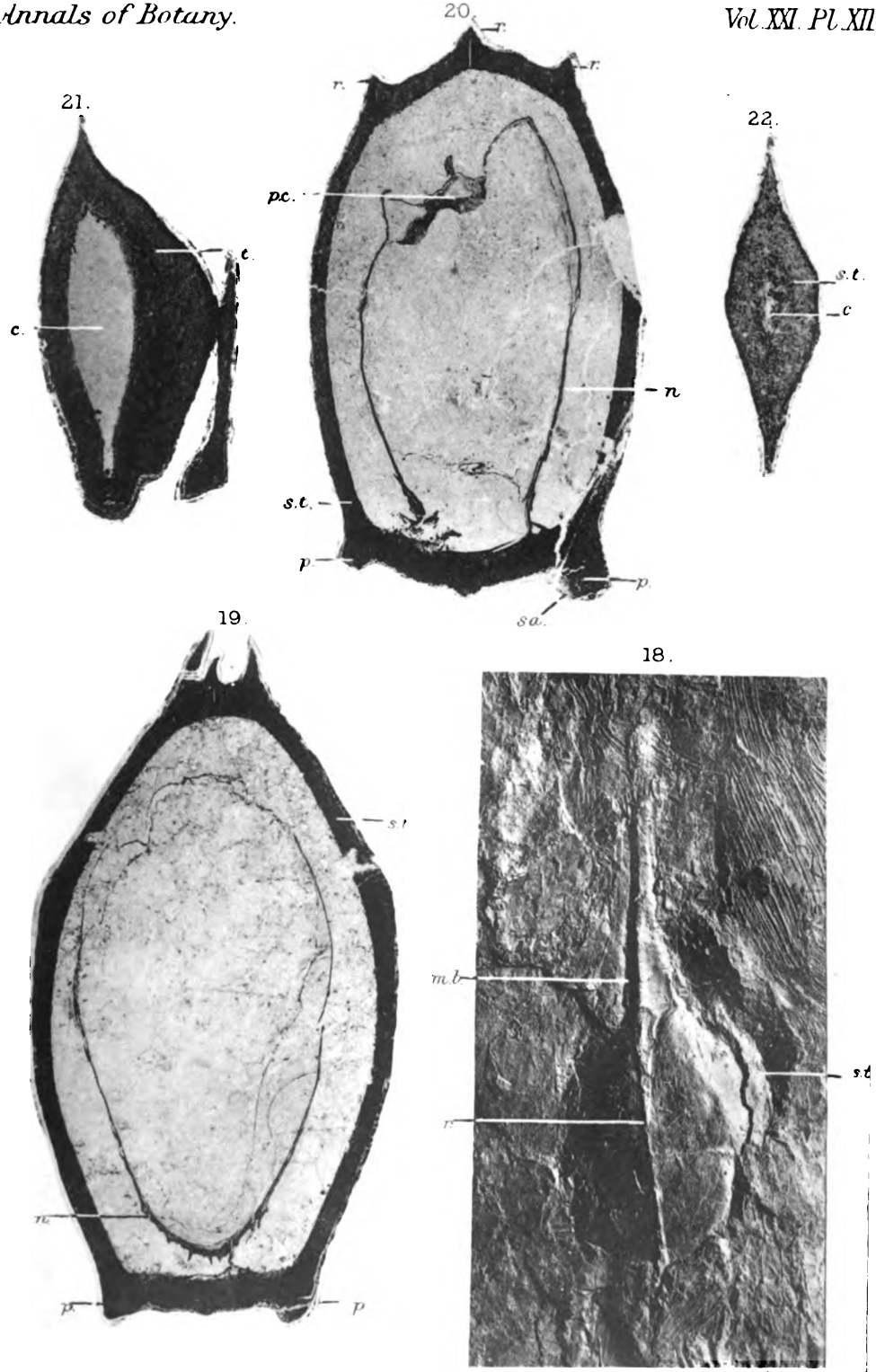

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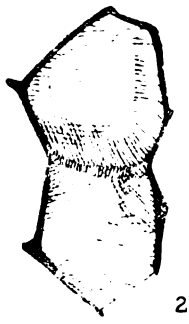

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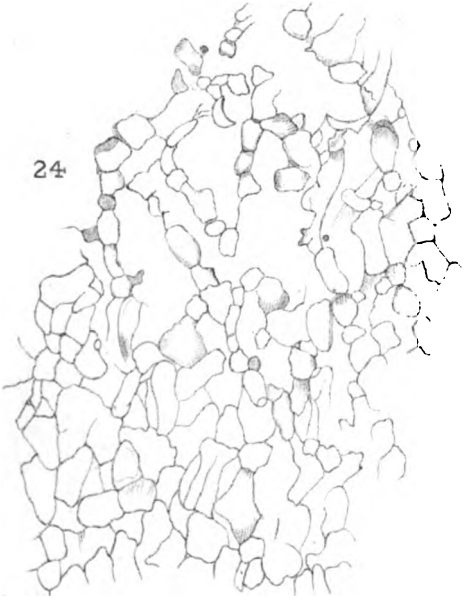

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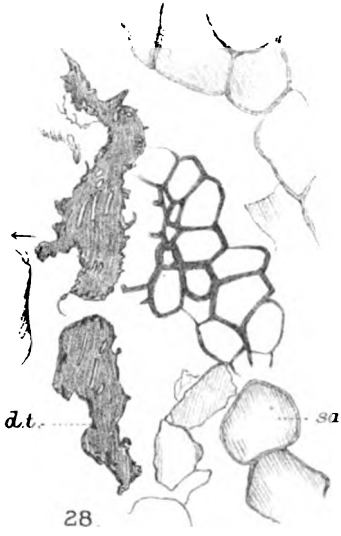

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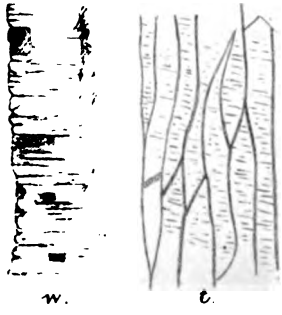

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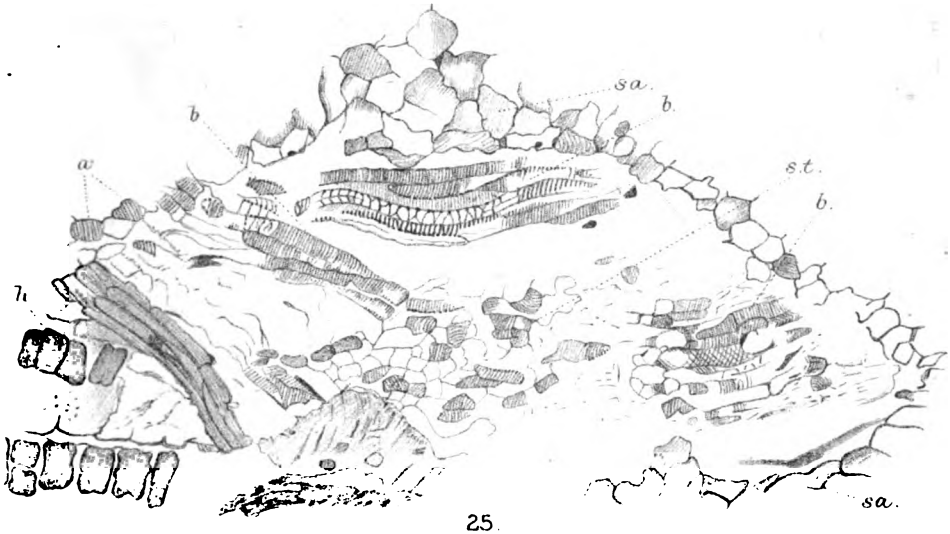

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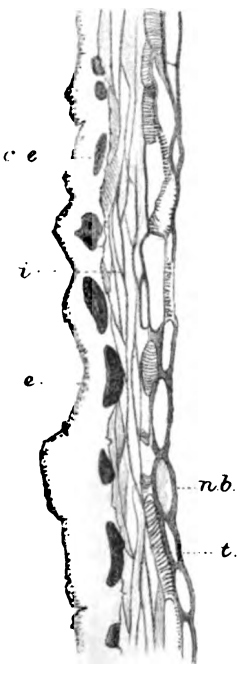

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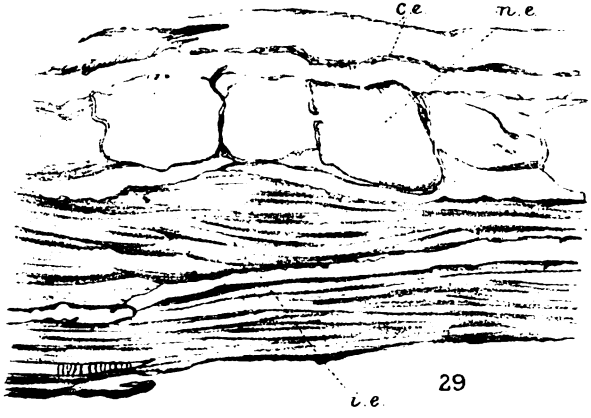

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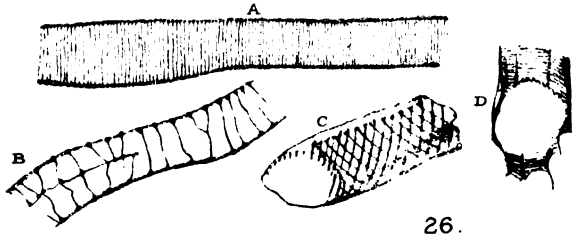

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NOTES.

THE DELAYED DEHISCENCE OF CALLISTEMON RIGIDA, R. BR.— This plant, commonly known as the stiff or rigid Bottle Brush, first described by R. Brown in the *Botanical Register*, 1819, Pl. 393, is frequently grown in Melbourne, although a native originally of West Australia. The tufts of red flowers at the ends of the branches are very striking in appearance, and leave behind sessile closely-set fruits which ultimately dehisce by three oval apertures in the centre of the flattened top of the semi-succulent fruit.

Since the plant flowers each year at the ends of the youngest branches only, the age of the fruit is that of the branch bearing them. Each fruit, sessile on the bark, is connected by a short stalk to the wood-cylinder of the year of its formation, the clusters of fruits leaving the wood perforated at such points by knot-holes. Hence the age of a fruit can be further verified by counting the number of annual rings inwards to the point of origin of its stalk.

When first formed the fruits are separated by spaces from one another, but since they grow at first relatively more rapidly in diameter than do the branches, they come into very close contact and remain so for the first two or three years. Ultimately they separate again, owing to the branch steadily increasing in diameter, while the fruits have practically ceased to grow. The same applies to the knots in the wood, which, after the fruits have fallen, are soon obliterated in the succeeding year's growths of wood. The fruits normally persist on the plant for many years, finally becoming rough and corky on the surface, though still containing living pericarp-cells, the pericarp-tissue being watery and pale green, with few scattered chloroplastids, more distinct in the younger fruits. These have no special cambial tissue except on the surface, where the layers of cork are formed, and on the stalk, where the latter passes through the cambium of the stem. The fruits nearly cease to grow in their second year (0·8 to 1 cm. diam.), the total increase in the next ten or even eighteen years being not more than 1 to 2 mm. The edges of the semi-succulent receptacle, however, which are at first nearly flat with the three carpellary valves, grow a millimetre or two upwards and inwards over the valves, sometimes nearly closing these in, but on drying always contracting so as to allow their dehiscence to set free the seeds.

Dehiscence and the escape of the seeds is normally delayed for three, four, or more years, and in one case portions of a cluster twenty years old were undehisced; the pericarp contained living and plasmolysable cells, and of the small linear seeds many also were found on microscopic examination to contain undoubted living cells. In the dry air of the laboratory, the fruits dehisced on cut branches, and the minute brown seeds fell in quantity. The same occurs in the open if an attached branch is

killed at its base by heat so that the water supply is cut off from the fruits, or even if the water supply is strongly diminished by partial interruption of the wood-cylinder, or by drought. Repeated attempts to germinate twenty-year-old and ten-year-old seeds failed, but it seems always to be a difficult matter to obtain satisfactory germination with the seeds of this plant.

The popular superstition is that the seeds will only germinate after subjection to intense heat, as after a bush fire. The origin of this idea probably lies in the fact that the fruits are retained undehisced for a long time, so long as they are supplied with sap, but that on dead or cut branches dehiscence soon follows as the result of the cessation of the supply of water. Scrub fires very commonly kill the basal ends of stems or branches, and leave the upper portions more or less uninjured, so that

FIG. 1. Clusters of fruits on stems of *Callistemon rigida*. (*a*) Cut and dried four-year-old stem, the fruits all dehisced. (*b*) Cluster twenty months old, freshly cut and fruits all undehisced. (*c*) Cluster nine months old, fruits half-grown and not yet in contact.

the still living fruits would then dehisce and shed their abundant seeds to germinate on the temporarily cleared ground beneath.

Dehisced fruits on living branches dry up, and fall gradually from the clusters, leaving these broken and irregular. Even when all have fallen, the cluster is still represented by pits on the bark which persist for some years.

We have therefore in this case a special instance of a plant which saves its seeds until the ground beneath has been cleared by fire or excessive drought, or until the branch or tree has been killed by these or other causes.

A fair-sized plant of this small shrubby tree, 12 to 15 feet high, may possess 1,200 clusters of fruits, each averaging fifty-two undehisced fruits, and the fruits containing approximately 250 seeds per valve, or 750 per fruit. The total number of seeds ready for dehiscence on such a tree might therefore amount to 40 or 50 millions. Hence it is hardly surprising that although the seeds are only about a millimetre long, by a fifth of a millimetre broad, the blackened ground beneath a tree whose base has been killed by a bush fire, while the upper branches are temporarily undamaged and living, should become completely covered by a brown layer of fallen seeds within a few days to a week or more.

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ON THE CONSTANCY OF CILIA-INSERTION IN BACTERIACEAE.— For the last eight months I have kept two forms of the genus *Pseudomonas* (cylindrical cells with polar cilia) under observation, constantly re-inoculating and causing them to become perfectly adapted to their environment. My object was to ascertain whether a form which had polar cilia could, under more favourable conditions, develop cilia all round the cell, i. e. become peritrich.

In the order Spirillaceae, on account of the undulating nature of the membrane, it is not to be expected that ciliation can be other than polar, and peritrich ciliation is never found: increase of motility, as I have previously observed, in *Spirillum gigan-teum* (syn. *Spirillum volutans*), is correlated with an increase in the number of polar cilia.

In the case of the Bacteriaceae, however, we are dealing with organisms whose membranes are stiff, and in which it is conceivable that increase of motion would be correlated with a development of cilia on the sides as well as at the poles. This would break down the distinction between the genus *Bacillus* and the genus *Pseudomonas*.

After eight months' cultivation of two forms of the genus *Pseudomonas* I have found that in all cases the cilia remained polar, even when better adaptation to the test-tube-culture environment, and consequently greater motility, had taken place.

Hence, to attain greater motility the cilia either become stronger or else more numerous at the poles only. In this they agree with the genus *Spirillum*.

DAVID ELLIS.

A Revision of the Geophilous Species of Peperomia, with some additional Notes on their Morphology and Seedling Structure.

BY

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With Plate XV.

THE discovery of the seedlings of certain of the geophilous Peperomias, which have been described in the last volume of the Annals of Botany [1], has necessitated a re-examination of all the bulbous species of the genus. The confusion existing between the Peruvian and Mexican representatives has been referred to, and some attempt has already been made to disentangle and distinguish the various species [2]. As it was impossible to clear up the various points of systematic interest from the material in our British herbaria, a visit has been paid to the herbaria at Geneva for this purpose.

I must here express my thanks to M. C. de Candolle for his kindness in allowing me to work in his Herbarium, where, in addition to his own collection, I was able to see all the material from the Berlin Herbarium, including the plants recently collected by Weberbauer and Fiebrig. He has further very generously allowed me to describe the new species, and to write this account of the geophilous forms, although he himself is at work on a new monograph of the genus. The Boissier and De Lessert Herbaria were also visited, and I am much indebted for the kindness and attention which I received there. I must also add a word of thanks to Dr. Stapf for the kind help he has given me in connexion with this revision. As the principal result of this work, a classification of the geophilous Peperomias is put forward on a new basis, namely, on the character of the underground tuber, which affords not only a very ready method of recognizing the plants from particular regions, but also appears to indicate, to a certain extent, the natural relationships of the various species.

The final separation of the species is effected mainly by the characters of the fruits, as in Dahlstedt's monograph [3], and in some cases the venation,

[1] Hill in Ann. Bot., vol. xx, p. 395. [2] Ibid., pp. 396–8.

[3] Dahlst. in Kong. Svenska Vet. Akad. Hand. Bd. 33, No. 2, 1900.

texture, and internal structure of the leaf are of great value. A few additional points of biological interest, with regard both to the seedling structure and to the development of the bulb or rhizome, have come to light during this investigation, an account of which will be given below. Seven new species are described in the course of this paper, and the descriptions of most of the known species have undergone considerable emendations.

History.

The first of the bulbous species to be described was the *Peperomia umbilicata* of Ruiz and Pavon[1] in 1798, from the neighbourhood of Lima in Peru; it is a well-marked form, which does not appear to have been brought to Europe since Pavon's time. The bulb or tuber is more or less placentiform in shape, the roots spring from the sides and base, whilst the upper part is bare and crowned by the rosette of radical leaves. The next specimens to arrive in Europe were those collected by Humboldt in Mexico, which are figured and referred by Kunth[2] in 1815 to the same species as Pavon's Peruvian plant. In the figure the roots appear to emerge more or less from a point on the upper surface of the bulb, which is described as being about the size of a pea.

In other respects the descriptions of the two plants agree, and since ripe fruits were not obtained, and the characters of bulb and leaf were not very clearly noted, it is scarcely surprising that these two plants should have been considered to belong to the same species. Since this time many geophilous forms have been collected both in Mexico and in Peru, and all those with a distinct bulb have been referred to *P. umbilicata*, apparently without any critical examination of the plants having been undertaken.

Thus in the Prodromus, at least four well-marked and easily separable species are included under *P. umbilicata*, R. and P., and even Dahlstedt, though he has removed certain plants from this collection to form his new species, *P. peruviana* (Miq.), Dahlst.[3], has failed to separate the Peruvian from the Mexican forms, with which they have been so long confused. Specimens belonging to two distinct species are also included under *P. parvifolia*, C. DC., both by De Candolle and Dahlstedt[4]. It is owing

[1] Ruiz and Pavon, Fl. Peruv., i, p. 30, T. 45, Fig. 6.

[2] H. B. and K., Nov. Gen., i, p. 59, T. 15, Fig. 1.

[3] Dahlst., l. c., p. 32.

[4] DC. Prod., XVI, i, p. 393. Dahlst., l. c., p. 30. *P. parvifolia*, C. DC. was described in Seeman's Journal of Botany of 1866, p. 133, from Pentland's specimen (from 12,850 feet) at Kew and from a Pavon specimen in the Boissier Herbarium. In the Prodromus in 1869, p. 393, Mandon's plant No. 1123 is added to the two just mentioned, and the diagnosis is amplified by a description of the ovary; the spikes also are said to be dense-flowered. The Pavon plant, which appears to be similar to Mandon No. 1123, has no ripe fruits, but was probably the plant on which the original description was based, for Pentland's plant is without doubt a specimen of *P. peruviana*, Dahlst.

to this confusion that the classification of the geophilous Peperomias has been so difficult, and it was in the hope of arriving at a solution of the problem that this research was undertaken.

General Morphology.

The correlation between the pseudo-monocotyledonous method of germination and the geophilous habit in these Peperomias has already been discussed [1], and some account of the underground tuberous portion of these plants has been given, but it will be useful to refer briefly to the various types of bulb or rhizome which are found among these species at this point, on account of their value for purposes of classification.

There appear to be four well-marked types of underground stem, which will be distinguished by the names of the prominent species. In the simplest or *parvifolia* type there is a simple, smooth, more or less spherical corm, with a basal tuft of roots and an apical crown of leaves [2]. In the case of old corms, owing to the division of the growing-point, there may be two or three rosettes of leaves. The central cylinder runs vertically through the corm from the stem-apex to the point of emergence of the roots, and the general appearance and structure very closely resembles that of a crocus corm. To this type belong four Peruvian and Bolivian species, *P. parvifolia*, *P. verruculosa*, *P. minuta*, and *P. cyclaminoides*.

To the next or *umbilicata* type belong those species in which the underground stem is a hypocotyledonary tuber, as in the preceding group, but the roots arise irregularly from the sides and base [3]. In the seedling [4] the primary root is vertical, but it is soon replaced by adventitious roots which spring from the sides of the little tubers [5]. The three species from South America, which must be included here, differ somewhat from each other. In *P. umbilicata*, R. and P., collected by Pavon, the roots arise in a somewhat regular ring round the middle of the spherical tuber, leaving the upper part free, and having a few roots scattered over the base; whilst in *P. peruviana* and *P. falsa* the roots are scattered in an indefinite manner over the sides and base of the tuber, which is often warted and irregular in outline [6].

The third or *campylotropa* type is of particular interest, and an account, with figures of the development of this peculiar form of tuber, has been given in the previous paper [7]. Some nine or ten species belong to this group and, with one exception, are all natives of Mexico and Central America. In the paper referred to, the opinion was expressed that this form of tuber was entirely confined to the Mexican region, but from an

[1] Hill, l. c., p. 397.
[2] Cf. Hill, l. c., Pl. XXIX, Fig. 27.
[3] Cf. Hill, l. c., Pl. XXIX, Figs. 1 and 2.
[4] l. c., Pl. XXIX, Figs. 5–10.
[5] l. c., Pl. XXIX, Figs. 11 and 15.
[6] l. c., Pl. XXIX, Fig. 1.
[7] l. c., pp. 407–410, Pl. XXX, Figs. 532–39.

examination of Gaudichaud's specimens from the country round Lima—with which one of Weberbauer's [1] plants agrees—it is clear that there is at least one South American example in which the roots have been carried round to the upper surface of the tuber [2]. This species, which I have named *P. Gaudichaudii*, has a somewhat different arrangement of its roots in old tubers to that seen in the Mexican examples. In the latter case the roots arise in a single tuft on one side of the stem-apex [3], whilst in the former (cf. Pl. XV, Figs. 3 and 4), although the bulb develops at first as in *P. pedicellata*, yet as it gets older the point of origin of the roots is gradually extended round the apex until the leaf rosette is enclosed by a ring of roots. These form a mass densely felted together by root hairs, which spread over and obscure the small bulb below, so that the leaves and inflorescences appear to spring from a dense mass of roots, and the bulb may be easily overlooked. Owing to the sharp curving of the central cylinder of the hypocotyl in the young tuber, by means of which the primary root is carried round to the upper surface [4], the name *campylotropa* has been given to Kunth's *P. umbilicata* from Mexico [5], which is perhaps the best known and most typical species of this group.

The mode of development of the underground stem in the fourth or rhizomatous type is known only in the case of *P. macrandra* [6], and it is possible that the three or four species, which can be placed in such a group, are not really very closely allied. *P. mexicana* occupies an intermediate position between the third and fourth types, since in the young plant there is a small warted tuber with a basal root, which appears to be carried round slowly to the upper surface; then by the continued growth of the stem-apex a short rhizome is produced, which may produce adventitious roots, but retains the old irregular tuber at its base (Pl. XV, Fig. 5) [7]. The effect in this case is, on a very small scale, like that produced by the rhizome of *Cyclamen europaeum* [8].

The typical rhizome of the group is seen in *P. macrandra* [9], where it occurs as a blackish-green, branched organ, creeping probably near the surface of the ground. It is some three or more cm. in length, with more or less erect branches about 1 cm. long, provided with adventitious roots, and marked by large leaf-scars. The mode of development of the two other species placed in this group is at present unknown. *P. monticola* appears at first to have a campylotropous bulb, which then develops

[1] Weberbauer, No. 1632 Amancäes, in montibus prope Lima 2-800 m.
[2] Hill in Ann. Bot. xx, footnote (1), p. 396.
[3] Ibid., l. c., Pl. XXX, Fig. 38.
[4] Ibid., l. c., Pl. XXX, Fig. 40.
[5] Kunth in H. B. and K., Nov. Gen., i, p. 59; l. c., p. 407.
[6] C. DC., in Ann. du Conserv. du Jard. Bot. Genève, 1878, p. 276.
[7] Hill, l. c., p. 411, Pl. XXX, Figs. 42, 44, 45.
[8] Hildebrand, Die Gattung *Cyclamen*, p. 22, &c.
[9] Hill, l. c., p. 412, Pl. XXX, Fig. 46.

into a short, irregularly tuberous rhizome; and *P. rupiceda*, C. DC., a new species brought by Weberbauer from Peru, has also a short, more or less horizontal, tuberous rhizome some 2 cm. long, provided with adventitious roots from all over the surface, and narrowed to the erect stem-apex.

It is possible that *P. puberula*, Baker[1], and some other species with well-developed rhizomes may also belong to the group, but until something is known of the seedling structure it is best to omit them from this revision.

The Leaves.

The leaves in these geophilous species are peltate, except perhaps in the case of *P. mexicana*, though in this species the peltate nature of the lamina is well seen in the aerial cotyledon. The petiole is inserted in a median position, as in *P. peruviana*, *P. campylotropa*, and *P. bracteata*, or more commonly its insertion is at a point at about one-third of the length of the lamina from the base. In outline the lamina may be orbicular, as in two of the species just mentioned, or the apex may be more or less pointed, giving rise to an orbicular-ovate or ovate shape, as in *P. bracteata*, *P. ovato-peltata*, *P. macrandra*, &c. The lamina is usually flat, but in the small-leaved, xerophytic species of the *parvifolia* group it is slightly concave.

There is a great difference in the texture of the lamina in the different species, and with this the character of the leaf venation is closely correlated. In *P. peruviana* the lamina is membranous when dry and the veins are very delicate; in a transverse section only a single layer of palisade tissue is seen. Similar membranous leaves are found in *P. ovato-peltata*, *claytonioides*, *macrandra*, and *gracillima*, &c. Nothing is known of their internal structure, but in these species it is evident from their appearance, and also from information supplied by the collectors, that they live in damp and shady places. A slightly stouter type of leaf, with a well-marked reticulate venation, is found in *P. umbilicata*, R. and P., and in *P. Gaudichaudii*. The texture of the lamina in *P. campylotropa*, *P. monticola*, *P. cyclaminoides*, and *P. rupiceda* is of a still firmer nature, being somewhat coriaceous, and the radiating veins can be easily seen, but the internal structure is not known.

The most highly specialized type of lamina is found in the *parvifolia* group, where it is very much reduced in size and increased in thickness. The small lamina is here usually orbicular-ovate or ovate, peltate about one-third of the distance from the base, and slightly concave. Of the three species concerned, *P. parvifolia*, *P. verruculosa*, and *P. minuta*, the internal structure of the leaf of the first two is known. In a transverse section two or three layers of palisade tissue are seen, below the water-storing tissue on the upper surface of the leaf, an arrangement correlated no doubt with the xerophytic conditions under which these species live.

[1] M. de Candolle tells me that he now considers *P. Mülleri* and *P. Bourgeaui* to be the same species and identical with *P. puberula*, Baker. Cf. Hill, l. c., p. 413 and footnote.

The Inflorescence[1].

The inflorescences of these small plants are usually numerous; they develop from the stem-apex and are surrounded by the leaf rosette. They consist of a peduncle bearing the flowers in a spike on its upper part, and may often attain to a considerable length. The lower or barren portion of the peduncle is in most cases about equal in length to the petioles, whilst a great deal of difference is shown in the character of the actual spikes in the different species. They may be short and crowded with flowers—some 5 mm. long in the *parvifolia* section—or, more commonly, they are long and lax flowered; usually simple, they are frequently branched in *P. ovato-peltata* and the allied species *P. claytonioides*, *P. pinulana*. The flower is like that found otherwise in the genus, and consists of two stamens and an ovary in the axil of a bract. The bract is peltate, and as a rule appears to be green, though in *P. ovato-peltata* it is white, and may be made use of as a specific character. In shape it is commonly ovate-acute or acuminate, and shows the same texture as the leaves, but in *P. bracteata* it is a fairly large and conspicuous orbicular structure which tends to fold round the spike. The anthers are either almost sessile, or are borne on short filaments ·4–·6 mm. in length. The fruits are often strikingly different in the different species, and afford the most certain characters for their determination. In most cases they are sessile or subsessile, but in the Central American species *P. pedicellata*, the stalk of the fruit is equal in length to the fruit itself. In shape the fruits are commonly globose or ovoid, and are often very small; they terminate either in a small dome-like style, bearing the stigma at its apex, or the style may be prolonged as in *P. peruviana*, *P. mexicana*, *P. macrandra*, &c., to form a conical or cylindrical beak, which in the case of *P. macrandra* is equal in length to the berry proper, and bears the stigmatic surface at its apex.

In the case of *P. rupiceda*, this stylar portion has extended backwards over the fruit to form a cap, covering the upper half of the globose fruit, and is quite unlike the style of any other geophilous species. The fruit-wall also is of a peculiar character, but since no seedlings have been found, it is at present uncertain whether this species really belongs to that subsection of the genus. The fruit-wall throughout the subsection affords some well-marked characters, which are of great value in the determination of the species; they may be arranged in two groups. In the one, the fruit-wall, as seen in surface view, is more or less smooth or slightly reticulated, and appears to be a somewhat delicate structure[2], whilst in the other, the wall is stouter and more resistant, being more or less deeply pitted, which gives the fruit a verrucose or verruculose appearance.[3]

[1] Hill in Ann. Bot. xx, Pl. XXIX, Figs. 1, 2, 27; Pl. XXX, Figs. 32, 42.
[2] Cf. Hill, l. c., Pl. XXIX, Fig. 3.
[3] Ibid. l. c., Pl. XXIX, Figs. 18, 29.

To the former type belong *P. peruviana*, *P. falsa*, *P. Gaudichaudii*, *P. ovato-peltata*, *P. claytonoides*, and *P. pedicellata* (Pl. XV, Figs. 9 and 11), whilst *P. parvifolia*, *P. verruculosa*, *P. cyclaminoides*, *P. bracteata*, and *P. campylotropa* (Pl. XV, Figs. 16 and 17) possess the more xerophytic type of fruit characteristic of the second group. The fruit of *P. monticola* (Pl. XV, Fig. 12) is quite distinct from either type, and will be described later.

In all cases the pericarp, which is some three to six cells in breadth, is bounded by a more or less thickened layer of cells, interrupted by pits, and it is these pits which give the surface of the fruit its characteristic appearance. In the group of which *P. peruviana* may be taken as a type, the walls of the external layer of pericarp cells are not much thickened, but the cells of this layer tend to be elongated tangentially, and the pits are broad and shallow. At the base of each pit there is a gland, consisting of a single cell with peculiar refringent contents [1]. The pericarp is usually about three to four cells broad, and the cells contain chlorophyll. When the seed is ripe the cell-walls are often found to have become blackened. The style in this group is usually a somewhat narrow conical or cylindrical organ bearing the stigma at its apex, and in *P. mexicana* and *P. macrandra* it attains to a considerable length [2].

In the xerophytic group, of which *P. parvifolia* is a good example, the pericarp is seen in section to be some five or six cells in breadth; the walls of the outermost layer of cells are more or less strongly thickened, and the cells themselves may be isodiametric or radially elongated [3].

In some of the species the cells of the outermost layer are more or less isodiametric, as in *P. minuta* (Pl. XV, Fig. 7), whilst in others they are radially elongated, e. g. *P. verruculosa* [4] and *P. cyclaminoides*. The innermost layer of cells next the testa is composed of large and tangentially elongated cells in some species. The glands at the base of the pits may be large single cells, as in *P. minuta* (Pl. XV, Fig. 7), *P. bracteata*, *P. campylotropa*, or they may be composed of a group of four or more cells, as in *P. parvifolia*, *P. verruculosa*, *P. cyclaminoides* (Pl. XV, Fig. 17), where they form conspicuous structures. The style in all these species is a small dome-like button at the apex of the fruit with the stigma at its summit. The fruit of *P. monticola* is peculiar in the black polished and faceted appearance of the surface of the pericarp. In section also it differs from the other geophilous species in the greater development of the pericarp, of which the basal part of the fruit is entirely composed (Pl. XV, Fig. 12). The outermost layer of the pericarp consists of thick-walled and very regular isodiametric cells, interrupted by narrow and not very deep pits, and this causes the peculiar faceted appearance of the fruit-wall.

[1] Hill, l. c., p. 400, Pl. XXIX, Fig. 4.
[2] Ibid., l. c., Pl. XXX, Figs. 43–47.
[3] Ibid., l. c., p. 405, Pl. XXIX, Figs. 19–20.
[4] Ibid., l. c., Pl. XXIX, Fig. 29.

Seedlings.

A few additional facts have come to light about the seedlings of two of the geophilous species. A fairly complete series of young plants of *P. Gaudichaudii* was found amongst Weberbauer's material, and seedlings and young plants of *P. macrandra*—the rhizomatous species—were found in the Boissier and De Lessert Herbaria.

In *P. Gaudichaudii* the development appears to be exactly similar to that described for *P. pedicellata*, although the youngest stages have not been seen. The roots, however, are much more numerous than in that species, and are freely branched, so that with their close felt of root hairs they form a dense mat over the surface of the tiny bulb (Pl. XV, Figs. 3 and 4). As the plant gets older the roots are gradually developed in a ring-like manner round the top of the tuber, and so encircle the stem-apex. The young seedling of *P. macrandra* has already been described [1]; the older stages now found are of interest, since they show that the rhizome is formed directly from the plumule by the slow upward growth of the stem-apex, and no curvature of the hypocotyl takes place as in *P. pedicellata*, &c. (cf. Pl. XV, Fig. 1). The figures of the young plants show the radicle at the base of the obovoid tuber with some secondary basal roots, and in the older stage two plumular leaves have developed from between the cotyledons at the apex of the tuber (Fig. 2).

Systematic Arrangement.

Turning now to the systematic arrangement of the various species, it is clear, in the first place, that they may be separated into two geographical groups, which have no species in common between them, although similar biological adaptations may occur in both areas. The following is a list of the species from the South American and Mexican regions, which, though in some cases imperfectly known, appear to belong to the geophilous subsection of the genus.

South American Species.

P. macrorhiza
P. scutellaefolia
P. umbilicata
P. Gaudichaudii
P. peruviana
P. falsa
P. parvifolia
P. verruculosa
P. minuta
P. cyclaminoides
P. rupiceda
P. cotyledon

[1] Hill in Ann. Bot. xx, p. 412, Pl. XXX, Fig. 48.

MEXICAN AND CENTRAL AMERICAN SPECIES.

P. ovato-peltata	*P. campylotropa*
P. claytonioides	*P. gracillima*
P. pinulana	*P. mexicana*
P. bracteata	*P. monticola*
P. pedicellata	*P. macrandra*

All the species appear to fall into the section (A.) *Eutildenia* of Dahlstedt [1], and they may be regarded as forming a distinct subsection, which for convenience will be termed *Geophila.*

It is, however, possible that some members of Miquel's subgenus *Panicularia* [2], as amplified by Dahlstedt [3], should be included with the geophilous species, although no evidence has been obtained from seedlings. *P. cotyledon* [4], particularly, which has a tuberous rhizome and peltate leaves, shows considerable external resemblance to species of the *Eutildenia* section, and the fruits are also of a similar type. The peculiar character of the compound spike or paniculate inflorescence therefore may have caused a separation of species which are really closely allied.

The position and characters of the subsection are as follows:—

	Peperomia.	
Subgenus V.	**Tildenia**	Miq.
Sectio A.	**Eutildenia**	Dahlst.
Subsectio I.	**Geophila**	A. W. Hill.

Tuber hypogaeum vel rhizoma tuberosum. Folia ad caulis basin rosulatim congesta, ± peltata, glabra. Bractea peltata. Planta novella (germinans) cotyledonibus longiuscule petiolatis heteromorphis; unius brevioris lamina minuta, hypogaea, in semine inclusa, quasi haustorium referens; alterius lamina parva, peltata, viridis, foliis assimilatoriis similis.

CLAVIS SPECIERUM.

A. Tuber hypogaeum basi radicibus instructum. Bacca ± ovoidea, apice scutulo late conico praedita. ¶ *Parvifoliae.*

(1) Lamina orbicularis, circa 1·5 cm. diametro, coriacea. Amenta 3–4 cm. longa. *P. cyclaminoides.*

(2) Lamina parva, ± ovata vel elliptica, carnosula. Amenta 4–8 mm. longa.

§ Lamina ± ovato-orbicularis.

† Bacca ovato-elliptica, verrucosa. *P. parvifolia.*

†† Bacca ovata, valde verruculosa. *P. verruculosa.*

§§ Lamina ± elliptica, minuta.

Bacca ovato-elliptica, minute punctata. *P. minuta.*

[1] Dahlst. in Kong. Svenska Vet. Akad. Hand. Bd. 33, no. 2, 1900.

[2] Miq., Syst. Pip., p. 117 [3] Dahlst., l. c., p. 49.

[4] Benth., Pl. Hartweg., in montibus Huacabamba, p. 148, Hartweg 833; cf. also *P. umbellata,* Miq., Chachapoyas, Peru; Matthews, No. 3230, Herb. Kew.

B. Tuber hypogaeum, basi lateribusque radicibus instructum. Bacca apice in appendicem producta. ¶ *Umbilicatae.*

(1) Lamina suborbicularis, ± subacuta, sub medium peltata. *P. umbilicata.*

(2) Lamina orbicularis, ad medium peltata.

§ Lamina tenuis. Bacca ellipsoidea, apice in appendicem angustam conico-cylindricam producta. *P. peruviana.*

§§ Lamina subcoriacea. Bacca globosa, apice in appendicem cylindricam producta. *P. falsa.*

Species non satis notae.

† Lamina ovata, sub medium peltata. *P. scutellaefolia.*

†† Lamina orbicularis, centro peltata. *P. macrorhiza.*

C. Tuber hypogaeum, campylotropum, radicibus fibrosis prope apicem ortis. ¶ *Campylotropae.*

(1) Bacca stipitata.

§ Bacca oblongo-cylindrica, stipite baccam ipsam ± aequante, brevistyla. *P. pedicellata.*

§§ Bacca cylindrica, breviter stipitata, longistyla. *P. mexicana.*

(2) Bacca subsessilis.

§ Pedunculus ramosus. Lamina ± ovata, sub medium peltata. *P. ovato-peltata, P. claytonioides, P. pinulana.*

§§ Pedunculus simplex. Lamina ± orbicularis.

† Lamina sub medium peltata.

Lamina ± membranacea, venis conspicue reticulatis. Bractea anguste elliptica, acuminata. Bacca globoso-ovata, subtiliter reticulata. *P. Gaudichaudii.*

†† Lamina ad medium peltata.

* Lamina tenuis, membranacea. Bractea ovato-acuta. Stamina filamentis instructa. *P. gracillima.*

** Lamina ± membranacea. Bractea conspicua, orbicularis. Stamina filamentis instructa. *P. bracteata.*

*** Lamina coriaceo-membranacea. Bractea ovato-acuminata. Antherae subsessiles. *P. campylotropa.*

D. Rhizoma tuberosum, ± repens. ¶ *Rhizomatosae.*

(1) Rhizoma repens. Lamina late ovata, acuta. Bacca elliptica, stipitata, in stylum cylindricum baccae aequilongum producta. *P. macrandra.*

(2) Rhizoma exiguum, vel tuber hypogaeum. Lamina ovata, acuminata. Bacca cylindrica, subtiliter stipitata, in stylum cylindricum dimidiam partem baccae aequantem producta. *P. mexicana.*

(3) Rhizoma tuberosum. Lamina ± orbicularis. Bacca nigra, verrucu-losa, ± globosa, apice pileo styloso obtecta. *P. rupiceda.*

(4) Rhizoma vel tuber irregulare. Lamina ± ovato-rotundata, subacuta. Bacca levis, nigra, cylindrica, apice rotundata; stigma sessilis. *P. monticola.*

(5) Rhizoma tuberosum. Lamina ± orbicularis. Amenta numerosa, in paniculam terminalem longe pedunculatam conferta. *P. cotyledon.*

¶ A. *Parvifoliae.*

P. cyclaminoides, A. W. Hill, sp. nov. (Pl. XV, Figs. 16 and 17).

Tuber hypogaeum, globoso-placentiforme more Cyclaminis, 1–2 cm. latum, ·5–1 cm. altum, basi radicibus fibrosis instructum. Caulis subnullus. Folia in rosulam congesta, in sicco luteo-viridia; petiolus 3–7 cm. longus; lamina orbiculari-ovata, apice obtusa, circa 2 cm. longa, 1·3–1·5 cm. lata, coriacea, sub medium ad ⅓ longitudinis peltata, venis magis minusve distinctis, circa 5. Amenta plura, 3–4 cm. longa, magis minusve laxiflora, pedunculis 6–8 cm. longis. Bractea ovato-acuta, carnosula, circa 1·1 mm. longa, ·7 mm. lata. Stamina filamentis parvis ·4–·5 mm. longis instructa. Bacca elliptico-globosa, 1·5 mm. longa, 1·2 mm. lata, verruculosa, apice scutulo late conico praedita. Semen elliptico-globosum, ·95 mm. longum, 8 mm. latum.

BOLIVIA AUSTRALIS. Pinos prope Tarija; 'In steriler feuchter Felswand im Moos,' 2,800 m. *Fiebrig*, No. 2488 (Herb. Berol.).

P. parvifolia, C. DC.

Tuber hypogaeum, nigrescens, magis minusve globosum, 1–1·5 cm. diametro, basi radicibus fibrosis instructum. Caulis subnullus. Folia in rosulam congesta, olivacea; petiolus 1·5–2·5 cm. longus; lamina magis minusve triangularis vel suborbicularis, circa 5–7 mm. longa, 5–5·5 mm. lata, apice obtusa vel rotundata, basi rotundata vel subcordata, sub medium ad ⅓ longitudinis peltata, carnosa, venis haud distinctis. Amenta plura, 4–5 mm. longa, densiflora, pedunculis, petiolis ± aequilongis. Bractea elliptica, acuta, circa 1 mm. longa, ·6 mm. lata, carnosula. Stamina subsessilia. Bacca ± ovato-elliptica, fulvo-olivacea, circa 1·4 mm. longa, 1 mm. lata, verrucosa, apice scutulo parvo late conico praedita. Semen ellipticum, circa ·8 mm. longum, ·65 mm. latum.

P. parvifolia, C. DC. in Seeman's Journ. Bot., 1866, p. 133; C. DC., in DC. Prod., XVI, i, p. 393; Dahlst. in Kong. Svenska Vet. Akad. Hand. Bd. 33, No. 2, 1900, p. 30, Tab. I, Fig. 7. A. W. Hill in Ann. Bot. xx, 1906, p. 404, Pl. XXIX, Figs. 18, 27, 28.

BOLIVIA. Prov. Larecaja. Vic. Sorata, Cabezeras de Chilcani, Lacatia, &c., 3,600–4,200 m., *Mandon*, No. 1123 (Herb. Kew, Herb. DC., &c.). Andes of Huanta; 12,000 ft. *Pearce* (Herb. Kew); *Bang*. No. 1860 —with aborted inflorescences (Herb. Berol.). Copacabana; circa 3,950–4,120 m. *A. W. Hill*, No. 181 (Herb. Kew). PERU. Herb. Pavon (in Herb. Boiss.). This plant appears to agree with No. 1123 of Mandon, but has no ripe fruits.

P. verruculosa, Dahlst., sp. nov.

Tuber hypogaeum, globosum, circa 1·2 cm. diametro, basi radicibus

fibrosis instructum. Caulis subnullus. Folia olivacea, in rosulam congesta; petiolus 1–2 cm. longus; lamina suborbicularis, apice basique magis minusve rotundata vel basi interdum subcordata, circa 6–8 mm. diametro, paullo sub medium peltata, subcucullata, carnosa, evenia. Amenta plura, 5–8 mm. longa, densiflora, pedunculis petiolis aequilongis. Bractea orbicularis, acuta, circa ·8 mm. longa, ·5–·6 mm. lata. Bacca ovata, fusca, circa 1·4 mm. longa, 1 mm. lata, valde et longe verruculosa, apice appendice obscuriore humili mammiformi medio stigmatifera praedita. Semen circa ·8 mm. longum, ·6 mm. latum.

P. verruculosa, Dahlst. ex A. W. Hill, Ann. Bot. xx, 1906, p. 406 (Pl. XXIX, Fig. 29).

PERU. Prope Sachshuaman, Cuzco, 3,700 m. *A. W. Hill*, no. 182. (Herb. Kew). Dep. Junin; inter Tarma et Oroya, 'Kalkfelsen,' 4,000 m. *Weberbauer*, no. 2544. Ad viam ferream Lima-Oroya, 'Hacienda Arapa-Yauli' (Porphyrfelsen), 4,400 m. *Weberbauer*, no. 305 (Herb. Berol.).

P. minuta, A. W. Hill, sp. nov. (Pl. XV, Figs. 6 and 7).

Tuber hypogaeum, depresso-globosum, 8 mm. diametro, 5 mm. altum, basi radicibus fibrosis instructum. Caulis subnullus. Folia olivacea, in rosulam congesta; petiolus 1–1·5 cm. longus; lamina elliptico-orbicularis vel elliptica, 3–3·5 mm. longa, 2–3 mm. lata, subcucullata, sub medium ad $\frac{1}{3}$ longitudinis peltata, carnosa, venis haud distinctis. Amenta plurima, minuta, circa 4 mm. longa, densiflora, pedunculis circa 2 cm. longis. Bractea late elliptica, carnosula, circa ·8 mm. longa, ·6 mm. lata. Stamina subsessilia. Bacca ovato-elliptica, fulva 1·5 mm. longa, ·8 mm. lata, minute punctata vel reticulata, apice scutulo parvo cylindrico praedita. Semen ellipticum, ·75 mm. longum, ·6 mm. latum.

PERU. Dep. Ancachs, Prov. Cajatambo; via inter Ocros et Chonta, cordillera nigra, 4,400 m. *Weberbauer*, no. 2776 (Herb. Berol.).

¶ B. *Umbilicatae.*

P. umbilicata, R. and P.

Tuber hypogaeum, lutescens, globosum vel ± placentiforme, circa 1·5 cm. diametro, lateribus et praesertim basi radicibus fibrosis instructum, supra nudum. Caulis subnullus. Folia in rosulam congesta, viridia, subtus albida; petiolus 4–6 cm. longus; lamina magis minusve orbiculata, saepius subacuta, 1·2–2 cm. diametro, submedium peltata, coriaceo-membranacea, venis reticulatis distinctis. Amenta 6–9 cm. longa, superne densiflora, inferne laxiflora. Bractea ovoideo-orbiculata, circa 1 mm. longa, ·7–·8 mm. lata. Stamina filamentis instructa. Bacca immatura, ± conica, ·8–·9 mm. longa, apice in appendicem parvam producta.

P. umbilicata, R. and P., Fl. Peruv., i, p. 30, Tab. 45, Fig. b.

PERU. In Coll. Limae; Chancay et Huanuci supra saxa, *Pavon* (Herb. Berol., Herb. Boiss.).

P. peruviana, Miq.; Dahlst. (descr. emend.).

Tuber hypogaeum, magis minusve globosum, fulvum, subere obtectum, basi lateribusque (ubique) radicibus fibrosis instructum. Caulis subnullus. Folia viridia, in rosulam conferta; petiolus 2–5 cm. longus; lamina orbicularis, 1–2 cm. diametro, ad medium peltata, tenuis, in sicco membranacea, venis tenuibus. Amenta 2–5 cm. longa, inferne ± laxiflora, pedunculis petiolis ± aequilongis. Bractea ovato-rotundata, circa ·8–·9 mm. longa, ·4–·5 mm. lata, apice satis longe attenuata. Stamina filamentis instructa. Bacca ellipsoidea, atro-viridis, circa ·7 mm. longa, ·5 mm. lata, minute reticulata, apice in appendicem angustam conico-cylindricam subobliquam circa ·3 mm. longam summo apice stigmatiferam producta.

P. peruviana, Miq. in Nov. Act. Nat. Cur., no. 19, Suppl., p. 483. Dahlst., l. c., p. 32, Taf. 1, Fig. 9. Hill, in Ann. Bot. xx, 1906, p. 400, Pl. XXIX, Figs. 1–17.

P. umbilicata, R. and P., l. c., C. DC., in DC. Prod., XVI, i, p. 393. Dahlst., l. c., p. 31.

P. parvifolia, C. DC. (Pentland sp.), in DC. Prodr., XVI, i, p. 393.

BOLIVIA. S. Francisco de Hay et Pisacoma; *Meyen* (Herb. Berol.). Prope L. Titicaca, 12,880 ft., *Pentland* (Herb. Kew). Prov. Larecaja; Vic. Sorata, collis Ullontigi, 2,800 m., *Mandon* 1,122 (Herb. Kew, etc.). Guaqui; 4,000 m. *A. W. Hill*, no. 180 (Herb. Kew). Austro-Bolivia; Tucumilla bei Tarija, *Fiebrig*, no. 2824[a] (Herb. Berol.).

ARGENTINA. Cienega; Sierra de Tucuman, 8,000 ft., no. 658, *Lorentz et Hieronymus*. Prov. Salta; N^do. Castillo, 12,000 ft., *Lorentz et Hieronymus* (Herb. Berol.). Prov. Salta; ex pampa grande, 2,800 m., usque ad limina nivis aeternae. Nevado de Cachi, 5,200 m., no. 12, *Speggazini* (Herb. DC.).

Forma *major*.

Petiolus 8–14 cm. longus; lamina orbicularis, 3–3·5 cm. diametro, e medio peltata, membranaceo-coriacea. Amenta circa 8 cm. longa, pedunculis ad 10 cm. longis. Bacca ovato-conica, ·9 mm. longa, basi ·5 mm. lata.

PERU. Ollantaytambo; prope Urubamba, 2,500 m. *A. W. Hill*, no. 183 (Herb. Kew).

P. falsa, sp. nov. A. W. Hill. Pl. XV, Figs. 10 and 11.

Tuber hypogaeum, irregulariter globosum, circa 1–2 cm. diametro, basi lateribusque radicibus fibrosis instructum. Folia viridia, in rosulam congesta; petiolus circa 3–5 cm. longus; lamina orbicularis, 1–1·3 cm. diametro, subcoriacea, e medio peltata, venis tenuibus. Amenta plurima, 1·5–3 cm. longa, ± densiflora, pedunculis petiolis ± aequilongis. Bractea orbiculari-acuta, circa ·9 mm. longa. Stamina filamentis minutis instructa. Bacca ± globosa, circa ·7 mm. longa, ·6 mm. lata, nigra, reticulata, apice in appendicem cylindricam pallide viridem circa 2 mm. longam summo apice stigmatiferam producta.

PERU. Pucara; inter saxa, 3,700 m. (Puno-Cuzco Via ferrea). *Weberbauer*, no. 451 (Herb. Berol.).

P. macrorhiza, Kunth in H. B. and K., Nov. Gen., v. 1, p. 72. Dahlst., l. c., p. 30.

PERU. Inter Coxamarca et Cerro de Centurcagua, alt. 1,800 m. (Herb. Berol.).

The specimen is too meagre to identify; the leaves are small, and the bulb is rather irregular in outline; the inflorescence is immature.

P. scutellaefolia, R. and P., Fl. Per., i, p. 29, Dahlst., l. c., p. 31.

PERU. In collibus ad Atiquipa.

¶ C. *Campylotropae.*

P. pedicellata, Dahlst. (descr. emend.).

Tuber hypogaeum, parvum, flavum, circa 5 mm. latum, 3 mm. altum, radicibus fibrosis instructum prope apicem ortis. Caulis subnullus. Folia viridia, pauca, in rosulam basalem disposita; petiolus 6–7 cm. longus; lamina ovata vel orbiculari-ovata 2·5–3 cm. longa, 2 cm. lata, basi leviter cordata, apice acuta vel ± acuminata, membranacea, septemnervia, prope basin peltata. Amenta valde laxa, pedunculis tenuibus petiolis longioribus. Bractea ovato-lanceolata, ± acuminata, circa 1 mm. longa. Stamina filamentis instructa. Bacca oblongo-cylindrica, fulva, subtiliter reticulata, longissime stipitata, stipite baccam ipsam ± aequante, circa ·8–·9 mm. longa, ·35 mm. lata, apice appendice conico-cylindrico praedita.

P. pedicellata, Dahlst., l. c., p. 35, Tab. i, Fig. 13. Hill, l. c., p. 408, Pl. XXX, Fig. 32, &c.

GUATEMALA. Santa Rosa; Dept. Santa Rosa, 3,000 ft., *J. Donnel Smith,* no. 3829 (Herb. Kew).[1]

P. mexicana, Miq. (descr. emend.).

Tuber hypogaeum, primum parvum, globosum, demum saepius basi lobatum, apice rhizoma exiguum formans, prope apicem radicibus fibrosis instructum. Caulis brevissimus, tuberosus. Folia viridia, in rosulam congesta; petiolus 1·5–2 cm. longus; lamina ovata, vel ovato-oblonga, acuminata ·5–2·5 cm. longa, 4 mm.–1 cm. lata, subtrinervia, in sicco membranacea. Amenta 4–5 cm. longa, ± laxiflora, pedunculis tenuibus circa 4–5 cm. longis. Bractea ovata vel ovato-lanceolata, ± acuminata, circa 1 mm. longa. Stamina filamentis instructa. Bacca cylindrica, fulva, circa 1 mm. longa, ·3–·4 mm. lata, subtiliter reticulata, breviter stipitata, stylo cylindrico dimidium baccae aequante apice stigmate capitellato terminato.

P. mexicana, Miq., Syst. Pip., p. 75, et in Nov. Act. Nat. Cur., 1846, p. 12, Tab. 6. C. DC., in DC. Prodr., XVI, i, p. 394. Dahlst., l. c., p. 34, Tab. i, Fig. 12. A. W. Hill in Ann. Bot. xx, 1906, p. 411, Pl. XXX, Figs. 41 and 42.

[1] Not in Herb. DC.; cf. Dahlst., l. c.

P. Galleotiana, Hooker, Ic. Pl. iv, Tab. 327.

Tildenia mexicana, Miq. in Diar. Inst. reg. Nederl., 1842.

MEXICO. *Kickx*; Miradores. *Linden*, no. 127 (Herb. Boiss.). Baranca de St. Francisco prope Mirador; in rupibus, *Liebman*, no. 97 (Herb. DC.). Cordillera Vera Cruz; *Galeotti*, no. 7111 (Herb. Kew, Herb. De Less. etc.).

P. ovato-peltata, C. DC. (descr. emend.).

Tuber hypogaeum, globosum, 1–1·8 cm. diametro, radicibus fibrosis confertis instructum, primum ex uno loco prope apicem ortis demum annulum circa rosulam foliorum formantibus. Caulis subnullus. Folia viridia, in rosulam disposita. Petiolus circa 6–12 cm. longus; lamina late-ovata, apice acuta, basi rotundata vel subcordata 5–8 cm. longa, 3–5 cm. lata, tenuis, sub medium peltata, venis circa 8. Scapus 12–18 cm. longus, simplex vel 2–3-ramosus, amentis ± densifloris. Bractea albida, elliptico-acuminata, circa 1·5 mm. longa. Stamina filamentis instructa. Bacca immatura, ovato-globosa, apice stylo conico-cylindrico baccae subaequilongo instructa.

P. ovato-peltata, C. DC. in Seem. Journ. Bot., p. 133, 1866, et in DC. Prod., XVI, i, p. 394; Dahlst., l. c., p. 34, Tab. i, Fig. 11.

P. umbilicata, R. and P., l. c. Dahlst., l. c., p. 31 (*J. D. Smith*, no. 1430).

COSTA RICA. San José; *Hoffman*, no. 521 (Herb. Berol.).

GUATEMALA. Dep. Baja Verapaz; Sta Rosa inter saxa humida, 5,000 ft. *H. von Türckheim*, no. 1430 (ed. J. Donnel Smith) (Herb. DC., Herb. Kew). *Nova Hispania*, *Pavon* (Herb. Boiss.).

A figure, attributed to this species, is given by Dahlstedt, in which the fruit is ovoid, with a small conical style not unlike that of *P. parvifolia*. The fruits of the specimens at Berlin are not quite ripe, but they are more or less globose with a fairly long cylindrical style, and appear to agree with those of *P. claytonioides*. It seems therefore probable that there is some mistake about Dahlstedt's figure.

P. pinulana, C. DC. (descr. emend.).

Tuber hypogaeum, placentiformi-globosum, circa 6 mm. latum, 4 mm. altum, radicibus fibrosis instructum, ex uno loco prope apicem ortis. Folia obscure viridia; petiolus circa 17 cm. longus; lamina subovato-rotundata, circa 3 cm. diametro, apice breviter obtusiuscule acuminata, basi repando-cordulata, carnosa, in sicco tenuissime membranacea, pellucida, ⅓ supra basin peltata, venis 9. Scapus ramosus, foliis circiter aequilongus; amenta cum pedunculis (1 cm.) circa 3 cm. longa. Bractea subovato-elliptica, apice subulata. Bacca ovata, apice in stylum contracta, stylo apice imo stigma globulosum puberulum gerens.

P. pinulana, C. DC. in Engl., Bot. Jahrb. x, p. 289.

GUATEMALA. Supra Pinula; prope Xalapa, ad truncos arborum in silvis; 1,800 m. *Lehmann*, no. 1693 (Herb. Boiss.).[1]

[1] Not in Herb. DC.

P. claytonioides, Kunth (Pl. XV, Figs. 8 and 9).

Tuber hypogaeum, globosum, circa 7 mm. diametro, radicibus fibrosis ex uno loco prope apicem instructum. Caulis subnullus. Folia supra laete viridia, subtus glaucescenti-viridia, opaca, in rosulam congesta. Lamina orbicularis vel ovato-orbicularis, 3–4 cm. diametro, apice acutiuscula, sub medium peltata, carnosula, venis 6–8. Scapus simplex vel 2–3 ramosus, ad 30 cm. longus; amenta laxiflora. Bractea ovato-acuminata, carnosula, centro viridula, ceterum albida, supra basin rotundata. Stamina filamentis instructa. Bacca globosa, ·8 mm. diametro, rugosa, apice in appendicem angustam cylindricam circa 4 mm. longam summo apice stigmatiferam producta. Semen globosum, ·65 mm. diametro.

P. claytonioides, Kunth in Ind. Sem. Hort. Bot. Berol., 1847, p. 11. C.DC. in DC. Prodr., XVI, i, p. 400. Dahlst., l.c., p. 33, Tab. i, Fig. 10.

GUATEMALA. Loco non indicato. *Sauer* communic. Specimina culta. H. Lefr. Montp. (Herb. Berol.). Ex Hort. Berol. (1847), (Herb. Berol.). Ex Hort. Kew, 1863 (Herb. Kew).

P. claytonioides is of considerable interest, since it appears to be the only geophilous species which has been raised from seed in Europe. In fact all of the specimens in our herbaria have been grown in different botanical gardens, and the original description of the species was founded on a plant raised in the gardens at Berlin. This species is very closely allied to *P. ovato-peltata*, as Dahlstedt[1] has already pointed out. It may, indeed, be identical with it, and possibly represents the cultivated form of *P. ovato-peltata*. In the shape of the leaves, the white inflorescence bracts and the fruits, which are globose with a style ·3–·4 mm. long, this species seems to agree with *P. ovato-peltata*. The most important points of difference are seen in the much larger and more delicate leaves of *P. ovato-peltata*, coupled with inflorescences which are shorter and less branched than those of *P. claytonioides*, both of which characters must be considerably influenced by environment.

P. pinulana is closely allied to both *P. ovato-peltata* and *P. claytonioides*, and Dahlstedt suggests that it may be only a variety of the latter. It seems to me more likely that both these species should be united to *P. ovato-peltata*, but the material is at present too scanty to justify such a union.

P. Gaudichaudii, A. W. Hill, sp. nov.

Tuber hypogaeum, parvum, globosum, ·5–1·2 cm. latum, 4–6 m. altum, radicibus fibrosis instructum, primum ex uno loco prope apicem ortis deinde rosulam foliorum cingentibus tuber obscurantibus. Caulis subnullus. Folia in rosulam congesta, olivacea; petiolus 2–8 cm. longus; lamina triangulari-orbicularis vel cordato-orbicularis, apice subobtusa, circa 5–9 mm. longa, 6–8 mm. lata, magis minusve membranacea, sub medium peltata, venis

[1] Dahlst., l. c., p. 34.

conspicue reticulatis. Amenta pauca, laxiflora, 6–8 cm. longa, pedunculis 2–6 cm. longis. Bractea anguste elliptica, acuminata, circa 1 mm. longa, ·4 mm. lata. Stamina filamentis instructa. Bacca globoso-ovata, circa 1 mm. longa, ·8 mm. lata, subtiliter reticulata, atrofusca. Semen ·75 mm. longum, ·65 latum.

P. umbilicata, R. and P., l. c. Miq., Syst. Pip., p. 70. C. DC. in DC. Prod, XVI, i, p. 393. Dahlst., l. c., p. 31.

P. peruviana, Dahlst., l. c., p. 33 (in parte).

PERU. Callao prope Lima, *Gaudich.* (Herb. Berol.). Lima, *Gaudich.* no. 150 (1834) (Herb. Berol., Herb. DC.). San Lorenzo, Lima, Callao, *Gaudich.* (Voyage Bonite, 1836). San Lorenzo, *Gaudich.*, no. 150 (1832) (Herb. De Less.). Lima, *Dombey*, *Boivin* (1839) (Herb. Boiss.). Amancaës; in montibus prope Lima, saxa in 'Loma formation' 200–800 m., *Weberbauer*, no. 1632 (Herb. Berol.). Dept. Junin, Prov. Tarma; Huacapitana ad Palca, 1900–2000 m. *Weberbauer*, no. 2013[1] (Herb. Berol.).

P. gracillima, S. Watson (descr. emend.).

Tuber hypogaeum, parvum, globosum, radicibus fibrosis instructum, prope apicem ortis. Caulis subnullus. Folia facie viridia, in dorso cuprea, in rosulam conferta; lamina magis minusve orbicularis, circa 1·5–2 cm. diametro, ad medium peltata, tenuis. Amenta cum pedunculis filiformibus circa 10 cm. longa. Stamina filamentis circa ·6 mm. longis instructa. Bacca immatura.

P. gracillima, S. Watson in Proc. Amer. Acad., xxii. (N. S., xiv), 1887, p. 448.

MEXICO. State of Jalisco; Rio Blanca, *Palmer*, no. 585 (Herb. Kew), in deep recesses of overhanging rocks.

Though the material is imperfect, this seems to be a distinct species, differing from *P. campylotropa* in the delicate membranous leaves and the stalked anthers.

P. bracteata, A. W. Hill, sp. nov. (Pl. XV, Fig. 15).

Tuber hypogaeum, orbiculare, circa ·8–1·2 cm. diametro, apice radicibus fibrosis instructum. Caulis subnullus. Folia viridia, in rosulam congesta; petiolus 8–10 cm. longus; lamina rhomboideo-orbicularis vel orbicularis, apice saepius paullo obtusa, 2–3 cm. diametro, fere e medio peltata, magis minusve membranacea, venis 8–9 conspicuis. Amenta 5–20 cm. longa, inferne laxiflora, pedunculis circa 10 cm. longis. Bractea viridis, magis minusve orbicularis, circa 1·5 mm. diametro; stamina filamentis instructa. Bacca globoso-fusiformis, 1·5 mm. longa, ·95 mm. lata, verrucosa, fulva, apice stylo cylindrico-conico praedita. Semen ellipticum, circa 1 mm. longum, ·8 mm. latum.

[1] This specimen probably belongs to this species, but the material is insufficient for a complete determination.

GUATEMALA. Dep. Huehuetenango; Sactos, inter saxa, *Cacc. et Ed. Seler*, no. 2731. 'Estancia de la Virgen' ad imam arborum, ibid., no. 2743 (Herb. Berol.).

P. campylctropa, A. W. Hill, sp. nov.

Tuber hypogaeum, placentiformi-globosum, circa 1–2 cm. latum, ·8–1·2 cm. altum, radicibus fibrosis instructum, ex uno loco prope apicem ortis. Caulis subnullus. Folia viridia in rosulam disposita; petiolus 3–8 cm. vel saepius 15–20 cm. longus; lamina suborbicularis, 1·5–3·5 cm. diametro, e medio peltata, magis minusve membranacea vel coriaceo-membranacea, venis 6–9 inferne distinctis. Amenta ± densiflora, cum pedunculis 8–20 vel interdum 30 cm. longa. Bractea ovata, acuminata, circa 1·5–1·7 cm. longa, ·8–·9 cm. lata. Stamina subsessilia. Bacca globoso-ovata, atro-fusca, circa 1·7 mm. longa, 1·2 mm. lata, verrucosa, apice scutulo late conico praedita. Semen globoso-ellipticum ·96 mm. longum, ·86 mm. latum.

P. umbilicata, Kunth in H. B. and K. Nov. Gen., I, p. 59, Pl. XV, Fig. 1. Hill, in Ann. Bot., xx, 1906, p. 407. Pl. XXX, Fig. 31.

P. umbilicata, R. and P., l.c. Miq., Syst. Pip., p. 70. C. DC. in DC. Prod., XVI, i, p. 393. Dahlst., l.c., p. 31.

P. umbilicata, var. *macrophylla*, C. DC. in DC. Prod., XVI, i, p. 394.

MEXICO. In locis excelsis scopulosis frigidis regni Mexicani, prope Sta. Rosa de la Sierra et Los Ioares alt. 2,600 m. *Humboldt* (Herb. Berol.); *Humboldt*, no. 764 (Herb. Willd.); *Uhde*, no. 253 (Herb. Berol.). Pédrégal; Vallée de Mexico 'among damp rocks with Ferns,' *Bourgeau*, no. 418. Santa Fé; Vallée de Mexico, *Bourgeau*, no. 631 (Herb. Kew, Herb. Boiss., Herb. DC.). State of Michoacan; 'damp hillsides' prope L. Patzcuaro, *Pringle*, no. 4124 (Herb. Kew, Herb. Boiss., Herb. Berol.). San Luis Potosi; 22° N. 6,000–8,000 ft., no. 802, *Parry and Palmer* (Herb. Kew, Herb. Boiss.)

P. sp. dubia; Mexico. *Graham*, 1830 (Herb. Kew). Mexico; Zunapan, *Coulter*, no. 1399 (Herb. Kew).

The plants included in this species differ considerably in the external morphology of their leaves. In the specimens collected by Pringle the leaf lamina is delicate, whilst in the Parry and Palmer specimens, &c., the lamina is fairly stout and rather coriaceous. In the characters of the bract, stamens, and fruit, however, there seems to be a fairly close agreement, so that the differences in general habit are probably due to the nature of the situations from which the various specimens have been collected.

¶ D. *Rhizomatosae.*

P. macrandra, C. DC. (descr. emend.).

Rhizoma hypogaeum, repens, circa 3 cm. longum, nigrum, ramis ± erectis circa 1 cm. longis, radicibus fibrosis instructum. Folia viridia; petiolus 10–15 cm. longus; lamina late ovata, acuta, 6–7 cm. longa,

4–4·5 cm. lata, ⅛ supra basin peltata, in sicco membranacea, venis 5–7. Amenta ad 13 cm. longa, inferne laxiflora, pedunculis ad 7 cm. longis. Bractea ovata, apice acuminata, fere e medio peltata. Stamina filamentis, circa 1·5 mm. longis instructa. Bacca elliptica, circa 1·2 mm. longa, breviter (·4 mm.) stipitata, in stylum cylindricum baccae aequilongum producta, summo apice stigma minute puberulum gerens.

P. macrandra, C. DC. in Ann. du Conserv. du Jard. Bot. Genève, 1898, p. 276. Hill, in Ann. Bot., xx, 1906, p. 412. Pl. XXX, Fig. 46.

MEXICO. Prov. Oaxaca; Sierra de San Felipe, 'wet ledges,' 8,500 ft. *Pringle*, no. 4654 (Herb. De Less., Boiss., Kew, Brit. Mus.).

P. monticola, Miq. (descr. emend.) (Pl. XV, Fig. 12).

Tuber hypogaeum, irregulariter globosum, vel rhizoma tuberosum, 2–4 cm. longum, circa 1–5 cm. diametro, supra saepius ubique radicibus fibrosis instructum. Caulis brevissimus vel subnullus. Folia atro-viridia, in rosulam congesta; petiolus 6–12 cm. longus; lamina rhomboideo-rotundata vel rotundo-ovata, apice subacuta, basi subcordata, circa 3–4·5 cm. diametro, e medio peltata, magis minusve coriacea, venis 7–9 conspicuis Amenta crassiuscula, ± densiflora, cum pedunculis 10–20 cm. longa. Bractea ovata, acuta, ·7 mm. longa. Stamina subsessilia. Bacca nigra, cylindrica, facie nitens, tessellata, circa 1·9 mm. longa, 1 mm. lata, apice basique rotundata, apice stigmate sessili praedita.

P. monticola, Miq. Syst. Pip., p. 71. Benth. in Pl. Hartweg, p. 293. Dahlst., l.c., p. 32.

P. umbilicata, *β. subacutifolia*, C. DC. in DC. Prod., XVI, i, p. 394.

P. umbilicata, *α. macrophylla*, C. DC. l. c.

MEXICO. *Galeotti*, no. 6023 (Herb. De Less.). Aguas Calientes; *Hartweg*, no. 1621 (Herb. Kew, Herb. De Less.); *Uhde*, no. 254 (Herb. Berol.). San Luis Potosi; *Schaffner*, no. 633 (Herb. Berol. ex Herb. Vigener). Convalli San Luis Potosi; in locis humidis, *Schaffner* no. 108 (Herb. Kew).

P. cotyledon, Benth. Pl. Hartweg, p. 148. C. DC. in DC. Prod., XVI, i, p. 401. Dahlst., l. c., p. 53.

PERU vel Ecuador. Huacabamba; in montibus, *Hartweg*, no. 833 (Herb. Kew, &c.).

Rhizome tuberous, peltate radical leaves. Inflorescence terminating in a short dense compound spike, with one or two whorls of leaves on the peduncle.

P. rupiceda, C. DC., MS. (sp. nov.) (Pl. XV, Figs. 13 and 14).

PERU. Lima. Oroya; inter Matucama et Tambo de Visa, 2,370–2,650 m. *Weberbauer*, no. 145 (Herb. Berol.).

Geographical Distribution.

The geophilous species form a very natural biological group, and their respective habitats seem to be fairly well defined. Too great stress must not be laid in all cases on the underground tuber as a guide to the relationships of different species, since the external features of any of these plants appear to depend largely on the conditions of their particular habitats. The *parvifolia* group, however, does seem to be a very natural one, for not only do the several species show affinities in numerous points, but they occur in a fairly definite line from about 10°–18° S. latitude. *P. minuta* is the most northerly species, and is succeeded by *P. verruculosa* in the regions from Oroya to Cuzco; *P. parvifolia* occurs around the southern end of Lake Titicaca, and *P. cyclaminoides*, which perhaps is not so closely related to the other three species, has been found in the mountains of Southern Bolivia near Tarija. They all show well-marked xerophytic characters, and apparently live in exposed places. Under such conditions the roots from the base of the bulb, growing more or less vertically downwards, would be able to obtain water from soil at some depth below the quickly drying surface.

The other South American species are more distinctly shade plants, with a fairly large leaf lamina, thin and membranous in texture when dried, and the walls of their fruits are delicate and apparently contain chlorophyll. Of the four well-known species of that group, *P. umbilicata*, *P. peruviana*, and *P. falsa* are no doubt closely allied. Their bulbs, with lateral and horizontally running adventitious roots, are similar in character. *P. Gaudichaudii*, however, in its campylotropous bulb, is a very distinct form, though in other respects it shows fairly close relationship to *P. umbilicata*. With the Mexican campylotropous species *P. Gaudichaudii* appears to have only a biological connexion, since it differs from them both in leaf and floral characters, and it seems most likely that a similar biological adaptation has arisen independently in the two widely separated localities.

Of the species just mentioned, *P. peruviana* appears to have the most extended range, namely from the vicinity of Urubamba in Peru in the north to the south of Bolivia, and to the province of Salta in the Argentine, whilst *P. Gaudichaudii* appears to be restricted to relatively low country (200–800 m.)[1] in the neighbourhood of Lima.

The Central American and Mexican species are recorded from Costa Rica (*P. ovato-peltata*), Guatemala (*P. claytonioides*, *P. pinulana*, *P. pedicellata*, *P. bracteata*), and from Mexico to about as far north as lat. 22° N. *P. monticola*, from the region of S. Luis Potosi, is the most northerly

[1] Weberbauer's No. 2013 from the Province of Tarma, Dept. Junin, 1900–2000 m. in the mountains above Lima, may perhaps belong to this species.

species. *P. campylotropa* and *P. gracillima* occur on the Pacific side of Mexico, whilst *P. mexicana* is found on the Atlantic side of the country in the Cordillera of Vera Cruz. *P. macrandra* from the province of Oaxaca appears to be the most southerly of the Mexican species.

All the species seem to be somewhat definitely localized, and with the exception of *P. monticola*, and perhaps some specimens referred to *P. campylotropa*, are all distinctly shade-loving plants. *P. bracteata*, and *P. campylotropa* to a much slighter degree, show some xerophytic characters in their verrucose fruits, but the leaves in these species are thin and membranous, and in some (e. g. *P. gracillima*) are very delicate.

The various species are in nearly all cases easily recognized and sharply defined, the bulbous habit and seedling structure being the principal points of similarity. *P. pedicellata*, *P. mexicana*, and *P. macrandra* show some affinity judging from their fruits and leaves, and *P. ovato-peltata*, *P. claytonioides* and *P. pinulana* form a very natural group, or perhaps they are only slightly different forms of the same species.

Of the remaining species there is little to be said. *P. monticola* is a very distinct form, and perhaps should not be included in this group. Of *P. campylotropa* and *P. gracillima* we have not sufficient material to point out their relationships.

Four new species of these geophilous Peperomias have been found in the last two or three years, and a careful examination of material, which has lain in Herbaria for many years, has revealed three or four distinct species hitherto confused with species already described. It seems, therefore, highly probable that there may be several species, as yet unknown to science, in the mountains of Central and South America, and a knowledge of their biological characters, seedling structure, and conditions of life would doubtless yield results of the highest interest.

EXPLANATIONS OF FIGURES IN PLATE XV.

Illustrating Mr. A. W. Hill's Paper on *Peperomia*.

The sections of the fruits are from photographs.

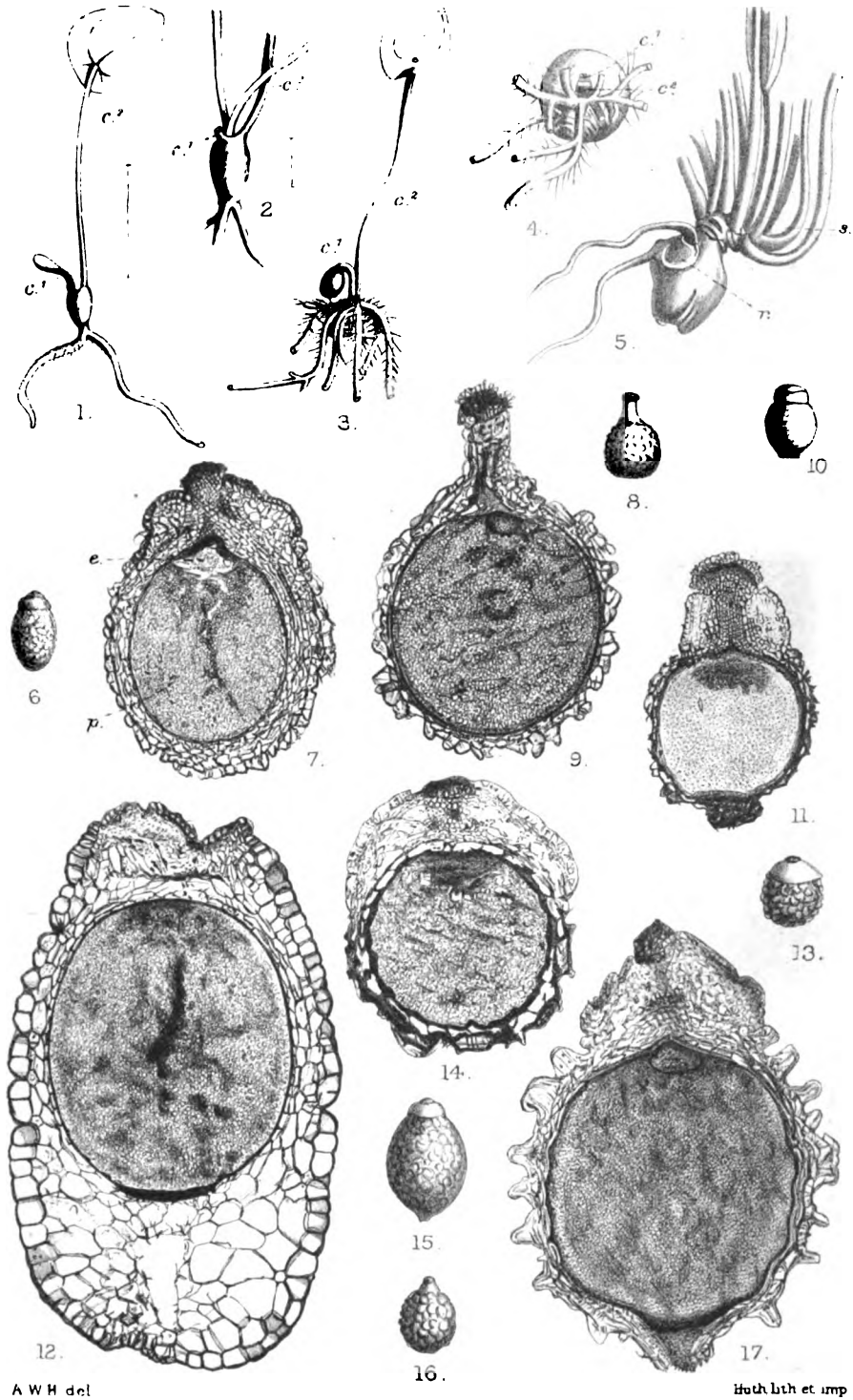
Fig. 1. *P. macrandra*, C. DC., a young seedling showing the laminae of both cotyledons and the tuber. c_1 = absorbent, c_2 = assimilating cotyledon.

Fig. 2. An older plant. Two plumular leaves have developed and the tuber has enlarged.

Fig. 3. *P. Gaudichaudii*, a young seedling, with its seed. The tuber is obscured by the closely felted roots.

Fig. 4. An older tuber seen from above, showing the two cotyledon scars and the point of emergence of the roots.

Fig. 5. *P. mexicana*, showing the development of the rhizome. The petiole bases only are shown.
Fig. 6. *P. minuta*, the fruit. × 9.
Fig. 7. The same in section. × 35.
Fig. 8. *P. claytonioides*, the fruit. × 10.
Fig. 9. The same in section. × 40.
Fig. 10. *P. falsa*, the fruit. × 10.
Fig. 11. The same in section. × 40.
Fig. 12. *P. monticola*, the fruit in section. × 40.
Fig. 13. *P. rupiceda*, the fruit. × 9.
Fig. 14. The same in section. × 35.
Fig. 15. *P. bracteata*, the fruit. × 10.
Fig. 16. *P. cyclaminoides*, the fruit. × 9.
Fig. 17. The same in section. × 45.



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HILL — PEPEROMIA.

Studies in Apospory and Apogamy in Ferns.

BY

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AND

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With Plates XVI–XX.

THE study of monstrosities has played a varying part in the growth of our ideas on morphology. At one time it has seemed as if teratology held the clues to the solution of all morphological problems, whilst at another time it has so sunk in estimation as to be looked on as little better than trifling and foolishness. One of the chief obstacles in the way of correctly weighing the value of teratological evidence lies in the difficulty of deciding, in any given instance, how far one is justified in reading a phylogenetic significance into an ontogenetic fact. It is probably true, for those who feel able to settle difficulties on *a priori* grounds—who belong to the school of transcendental morphologists—that 'Les monstruosités favoriseraient également tous les rêves de l'imagination, et . . . on verrait en elles tout ce qu'on voudrait y voir' (A. de St. Hilaire). But it is equally true that there is another and more profitable way of dealing with abnormalities. They may be regarded as the results of interference with ordinary reactions on the proper sequence of which that which we call 'normal' development depends. The introduction of experiment into teratological inquiry has done much to put the subject on a proper footing, and to encourage the hope that by its means we may be able to penetrate somewhat into the physiological workshops where morphological problems are constructed.

Amongst these problems none, perhaps, are of greater interest than those concerning sexuality. Two aspects of this matter have to be kept in view, firstly the facts and meaning of the sexual fusion itself, and secondly that correlative process of meiosis,[1] which is so intimately con-

[1] See Farmer and Moore, On the Maiotic Phase (Reduction-divisions) in Animals and Plants. Q. J. M. S., vol. xlviii, 1905. The terms 'Maiosis' and 'Maiotic' should have been written *Meiosis*, *Meiotic*, respectively. This form of the words is adopted in the present paper.

By the term meiosis, or meiotic phase, is meant that nuclear change which is concerned in the reduction in number of the chromosomes. Two mitoses are always involved, but the change in question

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nected with the former that it occurs as an intercalated phase somewhere in the life-cell cycle of every animal and plant that reproduces sexually. But the proper bearings, if any, of these cyclically recurring events upon morphological conceptions, e. g. that of alternation of generations, are still obscure, because we are at present in possession of so little of the evidence.

Considerations of this kind have strongly attracted us to the study of apospory and apogamy in Ferns, and especially towards the cytological aspects of the problem.[1] Many new facts have in the meantime been elucidated by workers in connexion with other groups of plants, but it seemed especially desirable that the Ferns, owing to the important position which the independence of the gametophyte and sporophyte generations respectively assures to them, should be closely investigated.

Since the discovery of apogamy by Farlow in 1874 and by De Bary in 1878, in various species of Ferns, our knowledge of the phenomena both of apogamy and of apospory has been greatly extended by the investigations of Druery[2] Bower[3] Lang[4] and others, who have not only increased the number of species in which the processes are known to occur, but have contributed much to elucidate the structural features involved.

The present paper will only deal with the results of our studies on the following plants, *Lastrea pseudo-mas* vars. *polydactyla*, Wills; *polydactyla*, Dadds; and *cristata apospora*, Druery. *Athyrium Filix-foemina* vars. *clarissima*, Jones; *clarissima*, Bolton; and *unco-glomeratum*, Stansfield. *Scolopendrium vulgare* var. *crispum Drummondae*.

We desire to express our indebtedness to Dr. Lang for apogamous prothallia of *Lastrea ps.-m. polydactyla*, Wills; to Prof. Bower for a fine plant of *Athyrium Filix-foemina* var. *clarissima*, Jones; and to Mr. Druery, who with unfailing generosity has supplied us with examples of all the other Ferns, and has assisted us in other ways from his fine collection of living plants. The actual cultivation of the material has been carried on at the Chelsea Physic Garden under the care of the curator, Mr. Hales.

probably always occurs in the first or heterotype division. The premeiotic number of chromosomes is that present between sexual fusion, resulting in the embryo, and the meiotic divisions in (the spore-mother-cells of) that embryo after it has become adult. Post-meiotic cells are those that intervene between meiosis and sexual fusion, thus they include all the *normal* gametophytic nuclei.

[1] Cf. Farmer, Moore and Digby, On the Cytology of Apogamy and Apospory. I. Preliminary note on Apogamy, Proc. Roy. Soc., vol. lxxi. Also Digby, Miss L. II. Preliminary note on Apospory. Ibid. vol. lxxvi.

[2] Cf. Druery, T. C., various papers in the Journal of the Linnean Society from 1884 to 1894, and elsewhere; also his Book of British Ferns, London.

[3] Bower, F. O., On Apospory and Allied Phenomena. Trans. Lin. Soc., 2nd ser., vol. ii, 1887. Also, On some Normal and Abnormal Developments of the Oophyte in *Trichomanes*. Annals of Botany, vol. i; and other papers.

[4] Lang, W. H., On Apogamy and the Development of Sporangia upon Fern Prothalli. Phil. Trans. Roy. Soc., vol. 190 (1898).

We propose to give a detailed account of each of the different plants separately, reserving a general discussion of the results for the conclusion of the paper.

1. *Athyrium Filix-foemina* var. *clarissima*, Jones.

The fronds of this Fern, as has been known since the investigations of Mr. Druery and of Prof. Bower, produce aposporous prothallia in connexion with the sporangia, which latter never in this plant reach maturity, but their peripheral cells grow out directly into prothallial filaments.

Two principal types of prothallium may be distinguished, firstly the filamentous or flattened form, which we shall speak of as the 'expanded' form, and secondly a more tuberous or fleshy growth, which we shall call the 'bulbous' prothallium (Fig. 17). The expanded prothallia were of far commoner occurrence in the conditions under which our cultures were carried on. They are delicate plants resembling ordinary prothallia with fimbriated edges, and bear antheridia and archegonia freely. But they nearly always fail to produce sporophytes, though we have succeeded in keeping them growing for several years.

The bulbous prothallia, on the other hand, are far more prolific in sporophytes, and nearly all of those we have succeeded in raising have originated from prothallia of this type.

Although the two types of prothallia are connected by intermediate forms, it will be convenient to treat their origin and structure separately.

A. *The Expanded type.* Professor Bower has given an account of the development in his memoir of 1887.[1] Our observations confirm his statements and in some slight degree serve to extend them. The sporangia generally begin to show signs of abortion soon after the archesporium is delimited, but frequent exceptions were encountered in which the arrest of development was delayed until after several archesporial divisions had occurred. Any peripheral cell of the sporangium, and sometimes of the stalk, may grow out as a prothallial filament, but those near the sporangial tip were most commonly the seat of the new growth.

The course of development is subject to some variation, but a filamentous structure is nearly always produced at first (Figs. 2, 3).

We have followed the development of these earlier stages with special care, as it was expected that it might be possible to trace in them nuclear changes which would indicate a transition from the sporophyte to the gametophyte. Every stage of the nuclear division has been seen, both in the developing sporangia and in the prothallial outgrowth up to the four-celled stage of the latter, and the result has been to confirm

[1] Bower, F. O., On Apospory and Allied Phenomena. Trans. Lin. Soc., new ser., vol. ii, 1887.

in every way the statement already made in a preliminary communication [1] to the effect that the transition from the sporophyte to the gametophyte in this Fern is attended by no reduction or alteration in the number of the chromosomes, nor is there any constant feature that would serve to differentiate these structures in the two generations respectively (cf. Figs. 8, 9, 13, 14, 15). We have further compared the chromosomes of the sporophytic tissue (Figs 9, 23, 24) in the embryo with the young cells of older prothallia with the same result.

It would be unsafe to predict that no case of chromosome reduction will ever be found to be associated with apospory, but at any rate it may be at once stated here that, so far as our present knowledge goes, apospory is always found to imply the absence of the meiotic phase from the life-cycle of the organism. And the natural corollary of this conclusion is that the embryos, when they occur on the 'gametophytes' of such plants, always arise apogamously, that is, they are formed without fertilization from cells or tissues that already possess the full complement of 'sporophytic' chromosomes which have persisted unchanged through that period of the life-history which is commonly termed the gametophyte.

The cell of the sporangium that is destined to a prothallium grows out in a papilla and its nucleus there divides (Fig. 8). The chromosomes at this division have been repeatedly counted, and they are approximately ninety.

It will of course be readily understood by those who are familiar with cytological work of this kind that an exact estimation is impossible, but we have taken every care to get as near as possible to the real number. In our former communication the totals were underestimated, owing to the great difficulty of distinguishing the individuals. It is easier to arrive at most consistent results by counting the chromosomes when in diakinesis, i. e. just before they congregate on to the spindle. The division is *typically premeiotic.*

The first division of the papilla most often results in the cutting off of a terminal cell (cf. Figs. 3, 6) that does not divide further, but forms a mucilage cell, whilst the more proximal cell by further divisions gives rise to the prothallium. It is important to note that all these mitoses are strictly of a premeiotic character, and no indication of a heterotype division is seen. A remarkable and constant character of the prothallial nuclei is afforded by the presence of several nucleoli often exhibiting curious forms (Figs. 10, 11, 12). They are quite diagnostic of the prothallia of this variety as distinguished from the type represented by *Ath. Filix-foemina*, and their presence affords a valuable criterion of the purity of cultures in the few doubtful cases that we have encountered. They are clearly 'chromatin nucleoli,' and their relation to the linin filament on approaching

[1] Digby, Miss L., loc. cit.

mitosis shows clearly that they are the chief sources of the chromatin, which at these periods of cellular activity becomes distributed in the thread. We have examined these nucleoli not only with stains, but also with other agents, e. g. pepsin, and this latter reagent indicates very clearly that they consist almost entirely of a substance unaffected by it. Incidentally, we may remark, that an acidulated solution of pepsin affords a valuable method which much facilitates the counting of chromosomes, as after such treatment they become much more distinct, owing to the removal of other proteid matter from the cell.

The prothallia, which usually show the customary differentiation of cushion and wings, produce large quantities of antheridia, and also a good crop of archegonia. The development of these calls for no comment, as it is perfectly normal up to maturity. In the antheridia, the mother-cells of the antherozoids were examined in order to test the possibility of a reduction occurring at this belated stage, but without any other result than to show conclusively that no reduction occurs. The antherozoids (which are of a larger size than those of the normal *Ath. Filix-foemina*) are vigorously motile, and exhibit chemiotactic response towards 0·1 % malic acid.

The archegonia, although they are normally formed, very speedily die. The nucleus of the oosphere sometimes fragments, but the whole central row of archegonial cells soon degenerates and assumes the familiar yellowish-brown colour so characteristic of archegonia that have failed to become fertilized. But although they are thus destined to abort, they nevertheless are able, at maturity, to exert an attractive influence upon the antherozoids.

On prothallia of this expanded type we have very seldom met with young sporophytes, and for a long time these were completely absent from our cultures. This appeared to us surprising, as Mr. Druery informed us that he was able to raise them readily. However, we did obtain a few, and these were always on prothallia that possessed a well-developed cushion. They are produced upon the cushion, and the superficial cells take part in the formation of the embryo. The root is formed endogenously, and burrows out of the prothallial tissue. It is impossible to distinguish in these cases the limit between embryo and prothallium, the cells of one pass insensibly (so far as appearance goes) into those of the other, and indeed, but for the presence of numerous archegonia in the cushion, it would be difficult to be sure that it was of prothallial nature at all and did not belong to the embryo itself. The embryo is, however, readily distinguished from one that has arisen (sexually or otherwise) from an oosphere by the absence of a foot. The transition from the tissues of one generation to those of the other is quite gradual, and the prothallium itself, as often happens in such cases, exhibits considerable tracheidal differentiation.

B. *The Bulbous Prothallium.* This type of prothallium seems to have

been met with by Mr. Druery, although his description indicates that his bulbils were not exactly similar to those produced by our plant. Probably the difference may depend on conditions of cultivation, for this type only became relatively frequent in our cultures when they were made in a cool greenhouse. They occur mixed with the expanded prothallia, and might be taken for vegetatively produced bulbils were it not for the fact that they frequently bear archegonia and antheridia. (The antheridia are, however, chiefly confined to such prothallia as occur along with them.) The bulbous prothallia recall the massive structures described by Bower in *Polystichum angulare* var. *pulcherrimum*, Padley, as one of the forms of prothallium produced aposporously on the leaves of that plant. They may be perhaps compared to the ordinary prothallia, by supposing that these had not developed wings, but had concentrated all growth upon the cushion. Probably the almost complete restriction of the embryo to the bulbous prothallium may be associated with storage of food.

It is often very difficult to make out the manner in which the embryo develops from these bulbils, but comparison of a very large series shows that the young sporophyte is differentiated gradually, so to speak, from the apex of the bulbil, and usually involves a larger or smaller number of the internal cells. Sometimes it is only the terminal cells of the bulbil that give rise to the young plantlet (Fig. 19), but oftener those of an axile strand are also concerned. The first leaf always appears as a direct outgrowth from the apical surface of the bulbil (Fig. 18), whilst the first root is formed endogenously. In some cases, especially when the bulbil has reached a relatively considerable size, the whole of the interior cells are modified to form vascular elements—tracheids and sieve-tubes—and a first impression is easily formed that the bulbil as a whole is really nothing but a vegetative sporophytic outgrowth from the parent leaf (Fig. 18). That this is not the case is always shown by more careful examination. The tissue connecting the bulbil with the leaf of the parent frond can invariably be made out to be prothallial in character, and this is further proved in the numerous instances in which archegonia, and sometimes also antheridia, have been found on its surface.

Of course the real nature of the bulbil is very important to determine, and we took every care to verify our conclusions as to this point, not only by microtome sections, but by examining entire bulbils after suitable staining and clearing.

The first stages of embryo formation (Fig. 19) are not easy to recognize, but they are betrayed by the existence of a little cap of whitish tissue situated on the somewhat broad free end of the bulbil.

Sometimes the embryo development begins very early in the history of the bulbil, and these cases have all been carefully examined, lest we might have been deceived by an example of vegetative sporophytic budding.

In no single instance did such vegetative reproduction of the sporophyte occur in our cultures.

No cases of migration of nuclei in the prothallial tissue of this variety have been seen.

The chromosomes of various parts of the embryo, taken from the young stem-leaf and root, have been counted, and the average numbers (90) very closely approximate to those obtained as the result of a study of the prothallial cells.

Cell Sizes. A remarkable difference exists between the cells of this plant and that of the type species (see below, p. 185). The cells and nuclei of the variety are very obviously of a larger size, and the same feature is also shared by the antherozoids (cf. Figs. 16 and 34).

Summarizing the results obtained from a study of this Fern, we may present them as follows:—

1. There are two types of prothallium, the expanded and the bulbous. The latter almost alone (in our cultures) bore sporophytes.

2. No change in the number of the chromosomes marks the transition from one stage in the life-history to another.

3. There is no migration of nuclei from one prothallial cell to another.

4. The nucleoli of the gametophyte are peculiar, and different from those of the prothallial nuclei of the type form.

5. The archegonia, although they are never fertilized, exert a chemiotactic influence upon the antherozoids which are very actively motile.

6. The embryo arises as a bud upon the gametophyte.

7. The cells, nuclei, and antherozoids of the variety each have a proper size which differs from that of the type Fern.

2. *Athyrium Filix-foemina* var. *clarissima*, Bolton.

This Fern was discovered in Lancashire by Mr. T. Bolton, who found it growing wild in 1892. It very closely resembles the preceding variety except that the ultimate branches of the leaves are somewhat spiral in character. The aposporous nature of the plant was established by Mr. Druery, who discovered that the prothallia were not only associated with the sori of (invariably) sterile sporangia, but that they might also occasionally arise directly from the apices of the pinnae.

Mr. Druery has twice given us fronds of this Fern, and they proved remarkably prolific, the prothallia appearing in crowds on pegging the frond down on damp soil. Unlike the preceding variety (*clarissima*, Jones), the prothallia produced by this plant are fertile to an extraordinary degree, and there is no difficulty in getting a large supply of embryos. We have grown some of the young sporophytes produced from the first lot of

prothallia, and these have again been used to provide a second generation. The plant has bred true with us, though Mr. Druery speaks of the progeny as being very inconstant.

The prothallia are nearly all of the expanded type, but a few cases of 'bulbous' prothallia have occurred in the cultures.

We have satisfied ourselves that, as in the preceding variety, there is no reduction of the chromosomes associated with any stage in the life-history of this Fern. The prothallial cells, and the antheridial cells up to the last division leading immediately to the formation of the spermatocytes, have been studied, but the number of the chromosomes remains unaltered (Fig. 26) and is identical with that obtained in the nuclei of the sporophyte (Fig. 27). The result of a large number of counts indicate that there are about eighty-four chromosomes. Of course, when they are so numerous, the numbers are not to be regarded as more than approximately correct; repeated countings of the chromosomes of the same nucleus often yield slightly different results. The limits of error, however, become narrowed by practice. It is more difficult to feel sure of the results of comparing together the nuclei of different varieties of this species of *Athyrium*. We have endeavoured to do this, and believe that there is a specific number for each variety. We find in practice that the number in the variety now under discussion is slightly inferior to that of var. *clarissima*, Jones (see p. 164), and somewhat more numerous than in the typical *Athyrium Filix-foemina*, in which they are about eighty. These numbers are obtained by averaging the results of reliable estimations, and they also coincide with the impression given by a close examination and comparison of the nuclei of the varieties in question.

The prothallial nuclei also exhibit the same remarkable chromatin nucleoli (Fig. 25) that are so striking in var. *clarissima*, Jones, but they are not so prominent as in that variety. In fact, in this as in many other respects, Mr. Bolton's Fern stands about midway between the type form and Col. Jones's plant.

The prothallia, as has been observed, are extremely fertile. They produce large crops of antheridia, especially on the smaller prothallia, whilst upon the cushions of the larger ones archegonia of perfectly normal appearance are formed. As an exceptional circumstance, both antheridia and archegonia may occur on the upper surface of the prothallium, but they are for the most part restricted to the lower surface.

The antherozoids are not as large as those of the *clarissima*, Jones, and differ in no constant character from those of the typical plant. The archegonia are formed in the normal manner, and when the neck opens the antherozoids are chemiotactically attracted and crowd into it, a few often slipping down into the venter. But no act of fertilization occurs, although, as will be seen, the embryo originates from the oosphere itself.

The antherozoids seem generally to be attracted as far as the ventral canal cell, and only accidentally to reach the actual cavity of the venter (Figs. 28, 29), though occasionally as many as three or four were seen there. They do not enter the egg, which is surrounded by a delicate pellicle. The antherozoids soon die after they reach the venter.

Whether or not any stimulus to further development on the part of the oosphere is given by their proximity must be left undecided. The two kinds of sexual organs are produced in such large numbers that it has not been practicable to isolate prothallia-bearing archegonia, but the indirect as well as the negative evidence against impregnation seems to us to be conclusive. Not only have we not seen anything to indicate the entrance of an antherozoid into an egg, but the absence of any increase in the chromosomes in the embryo, as compared with the prothallial cells, would seem to preclude the possibility of any act of fertilization having occurred. As in the foregoing variety, no migrations of the nuclei from one prothallial cell to another were seen.

The embryo in this variety, so far as our own material is concerned, invariably arises from the oosphere (Fig. 29). In this respect it stands in sharp contrast to that of the preceding variety, in which it as constantly originates from prothallial tissue, and never directly from the egg. The first divisions are somewhat inconstant, but the basal wall, parallel to the long axis of the archegonium, is the first to appear. The two halves of the embryo then segment differently, and this is perhaps to be correlated with the blunter form of the anterior and the more pointed character of the posterior half of the embryo at this stage. In the epibasal half (Fig. 30), the median wall almost always precedes the appearance of the transversal wall, but these two walls cut up the epibasal half of the embryo into the usual four octants. In the hypobasal half of the embryo the first division-wall is most commonly *parallel* to the basal wall, and thus in longitudinal sections the embryo as a whole appears to consist of a row of these cells (Fig. 29). Then each of the two cells in the hypobasal region is divided by a wall most often situated in the median plane. It may happen, however, that the transversal wall appears instead of the median, though this appears to be exceptional.

We did not attempt to follow out the further development of the embryo in detail. The process very closely resembles that of an ordinary Fern. A tolerably well-developed foot is formed, though it is smaller than the corresponding organ in the typical forms. The cells surrounding the base of the archegonial cavity become filled with temporary reserve materials which are ultimately absorbed, on behalf of the growing embryo, by the cells of the foot.

As in the preceding variety, there is a very regular and characteristic difference in the average size of the cells and nuclei as compared with those

of the type species. In this, as in many other characters, the present form stands about midway between the type and Col. Jones's variety. It is difficult to give exact ratios, as the sizes of individual cells vary a good deal, but by making drawings in outline of the cells and nuclei of corresponding regions of prothallia and leaves, both of the varieties and of the typical plant, we have arrived at approximate values. We shall give the data in another paragraph (see p. 185).

It is clear that in the case of *Ath. Filix-f. clar.*, Bolton, we meet with an example of what, in the absence of the aposporous origin of the prothallium, would be regarded as parthenogenesis, for, as in true parthenogenesis (if it really ever occurs), the egg gives rise to an embryo without fertilization. But in this instance the egg itself is abnormal in respect of its nuclear composition. The nucleus of a normal oosphere produced on an ordinary prothallium only possesses half the number of chromosomes characteristic of the sporophyte. That is, meiosis has intervened (at the spore-mother-cell divisions), and the premeiotic number of chromosomes is no longer present, but has become halved, owing to the peculiar mode of distribution of entire chromosomes (the allelomorphs of each pair) at meiosis. But in the case before us the meiotic phase has been omitted, and consequently the *premeiotic* number of chromosomes characteristic of the sporophyte is retained throughout the gametophyte and is present in the oosphere. The egg then cannot be regarded as the exact physiological equivalent of that produced by a normal prothallium. It already possesses the full number of chromosomes that normally would only be provided as the result of fertilization. Hence from the point of view of the nuclear constituents—at any rate as regards the chromosome contents—the act of fertilization would be superfluous, and indeed, unless some method of regulating the number of chromosomes other than by meiosis should become operative, it would be contrary to expectation. The possibility, however, of such regulation should not be overlooked, in the light of Němec's statements respecting the restoration of the normal number when this had been artificially modified, as in his experiments on anaesthetized roots. On the other hand, the study of many animal embryos, in which the number of normal chromosomes had in various ways been disturbed, clearly indicates that such a power of regulation is exceptional. Indeed it may be stated generally that, whatever number an embryo possesses on starting development, this is retained as far as development proceeds. The few exceptional cases, resulting from polyspermy, only emphasize the individuality of the chromosomes. A survey of the facts at present available renders it difficult to see how an egg that had retained the premeiotic number of chromosomes could possibly become fertilized.

We may summarize the facts connected with the development of *Ath. Filix-foemina* var. *clarissima*, Bolton, as follows:—

1. The prothallia are both soral and (very rarely) apical, and the expanded type greatly predominates over the bulbous form.

2. Sexual organs are very freely produced, and the antherozoids are very active, and are chemiotactically attracted by the archegonial contents, some of them even reaching the venter.

3. No true fertilization occurs.

4. No migration of prothallial nuclei were observed.

5. The egg develops apogamously, and in its earlier ontogenetic stages follows (though not exactly) the ordinary Fern type.

6. The cells of the sporophyte and of the prothallium are larger than those of the type Fern, and smaller than those of the var. *clarissima*, Jones.

3. *Athyrium Filix-foemina* var. *unco-glomeratum*, Stansfield.[1]

We owe material of this plant to the kindness of Mr. Druery, who in 1904 gave us part of a frond, which on being pegged down produced aposporous prothallia, both from the sori and apices of the pinnae. Two sporophytes were raised, one of which 'reverted' to the ordinary type of *A. Filix-foemina*, whilst the other one kept true to the varietal form. It produced the aborting sori during the summer of 1906, and from these fronds we have obtained enough material to work out the general outlines of development, though not in full detail. We propose to investigate this form further, and also the future behaviour of the descendants of the reverted plant.

The prothallia spring, as has been said, from the abortive sporangia and also from the apices and margins of the frond, which are notably pellucid in texture. The sporangia are not numerous, and they are remarkably elongated; the prothallia commonly springing from the apical (or terminal) cell. Like those of var. *clarissima*, Jones, the prothallia are of the expanded and bulbous types, though the latter are not so markedly different from the former as in that variety. They resemble rather a prothallium that has been almost reduced to a cushion, but they often bear filamentous outgrowths. The expanded prothallia commonly possess fimbriated edges, and this peculiarity (also shared with *clarissima*, Jones) is to be associated with numerous adventitious marginal growing-points of limited duration. Such new growing-points may even arise from the marginal cells of the sinus close to the principal apical cell. Archegonia and antheridia are both produced. We have not succeeded in raising enough sporophytes to clear up the embryogeny. But it is certain that the embryo arises endogenously and in connexion with an archegonium, and that it possesses a somewhat rudimentary foot. These facts indicate

[1] Cf. Stansfield, F. W., On the Production of Apospory by Environment in *Athyrium Filix-foemina* var. *unco-glomeratum*. Journ. of the Lin. Soc., vol. xxxiv.

that the process resembles that of *clarissima*, Bolton, rather than that of *clarissima*, Jones.

One of the most striking features of the prothallia of var. *uncoglomeratum* is the very large size of the cells and the nuclei. They are far larger than in any of the other varieties of *Athyrium* with which we are acquainted, and the nuclei share with the preceding varieties the existence of well-marked and numerous nucleoli (Fig. 31). The antherozoids, similarly, are the largest we have met with amongst these varieties of *Athyrium*.

The number of the chromosomes remains constant through the life-history, no reduction taking place in connexion with the gametophyte. The actual numbers are very difficult to decide, but we estimate them at about 100. They are certainly more numerous than in the other varieties we have examined.

As in the other varieties of *Athyrium* here dealt with, there is no indication of nuclear migration in the prothallium.

4. *Scolopendrium vulgare* var. *crispum Drummondae*.

This fern was given us by Mr. Druery. It was first recognized as an aposporous variety, and as such described by him in 1893[1]. The prothallia arise in great profusion, especially from the margins of the frond, and sometimes they occur even before the frond is pegged down on the soil. They are somewhat irregular in shape, but all belong (so far as our experience goes) to the expanded type. Antheridia and archegonia are freely produced, and are formed on both surfaces of the prothallia, though more abundantly upon the lower surface. We have raised young plants to test their purity, and find that the variety breeds true on the whole, though there is some latitude in the 'crisp' character of the fronds.

The chromosomes are very numerous, but they follow what we have found to be the rule in all the aposporous ferns which we have studied. That is, there is no cyclical alteration in the number of chromosomes; but we found a larger difference in the numbers given by various countings than we have met with in most of the other ferns,[2] and we are inclined to think these variations probably are not due entirely to errors of estimation, but do really correspond to fluctuations in the numbers actually present in the different cells. Thus we found in the embryo a considerable number of cases in which the chromosomes were about 95–100, but two instances in which we could only distinguish about 80. In the prothallial cells the average number was 70, whilst in the archegonial nuclei only 80–83 chromosomes could be identified. The antheridial nuclei are difficult to deal with on account of their small size, but in those cases on which we

[1] Journ. Lin. Soc., xxx.

[2] Cf. however the results on pp. 182, 188 for *Lastrea pseudo-mas* var. *cristata apospora*.

could at all rely, the numbers obtained varied between 70 and 82. Whilst it seems certain, then, that there is no reduction in the accepted sense of the term, there is evidence of a slightly larger number of chromosomes in the sporophyte as contrasted with the gametophyte. Too much weight must not be placed on this, and there is another circumstance which should be mentioned in this connexion that seems to us to indicate a possible explanation of the irregularities that we have encountered, not only in this, but in the other forms. A study of the common *Scolopendrium vulgare* of the typical form shows that it contains a much smaller number of chromosomes than does the variety. We find that in the spore-mother-cells, when they divide, the reduced (meiotic) number of chromosomes is 32, thus confirming Stevens's[1] and Gregory's[2] statements for this species. The number in the premeiotic cells of the sporophyte of the same plant is 64. It is clear then that these must have broken up into smaller units, or have been replaced by such, but it is also obvious that this multiplication has not resulted in the production of exact multiples in the variety of the chromosomes present in the original parent. Thus it is not inherently improbable that individual differences may exist, and that the different cells may lack uniformity in the matter of their chromosomes.

Scolopendrium vulgare is remarkable for the number of diverse forms to which it has given rise, and we propose to examine these further in order to test the hypothesis that the variation in form may be associated with fluctuation in the number of chromosomes. It is not improbable that *Scolopendrium* and its variety only represent a comparatively extreme example of chromosome-variation, and that the reason why one fails to arrive at the same conviction with regard to the existence of variation in the preceding ferns may be merely due to the divergence from the mean being so small as to fall within what we regard as the limits of error in observation.

The archegonia, as has been already stated, are produced on a fairly extensive cushion in great numbers, and they occur on both surfaces of the prothallium. Embryos are formed apogamously, and they originate, as in *Athyrium Filix-foemina* var. *clarissima*, Bolton, from the oospheres.

The archegonia develop quite normally, and we have been able readily to follow all the stages; these being diagrammatic in the clearness with which they can be distinguished. After the ventral canal has been cut off from the oosphere the latter body assumes the well-known bun shape, with a slight anterior depression. The nucleus is of the usual basin-like form, and in the hollow can readily be seen the corpuscle or 'body'[3] that is a regular feature of the normal oospheres.

[1] Stevens, Ber. d. Deutsch. Bot. Gesellsch., 1898. [2] Ann. Bot., xviii, p. 447.

[3] This 'body' lies sometimes in the 'receptive spot'; sometimes, though more rarely, outside it.

We have studied the process of fertilization in the common Male Fern (Fig. 35) for purposes of comparison, and it is easily seen that the similarity between the oosphere in our *Scolopendrium* and that of a normal Fern ends as maturity approaches. Before the neck of the archegonium opens, in many cases at any rate, the egg becomes surrounded with a membrane, and the possibility of fertilization is thus definitely excluded. It is very common in old degenerating archegonia to find the oospheres thus enclosed in a proper membrane, whilst otherwise exhibiting signs of disintegration. A frequent symptom of approaching death is visible in a more or less complete fragmentation of the nucleus. We had observed in otherwise healthy and comparatively young oospheres (Fig. 36), which had not yet become surrounded with membranes, that occasionally there were two well-defined and large nucleoli, and that the linin was clearly grouped with reference to these two centres. In other cases it appeared as if amitosis were going to supervene, for the nucleus had become correspondingly two-lobed, connexion by means of a very narrow isthmus being, however, preserved. But we are convinced that these appearances are signs of approaching senility, and that they do not represent stages in the first segmentation of the egg. Of course, the definite proof of this can only be given when the first division is actually seen, and we have as yet not succeeded in obtaining preparations to illustrate this, but the weight of probability is strongly in favour of an ordinary mitosis being associated with the first, as it is with the later divisions of the embryo.

The mode of segmentation of the embryo, more than one of which may be formed from one prothallium, closely follows that already described in *Ath. Filix-foemina* var. *clarissima*, Bolton. The first wall, the basal, separates the epibasal from the hypobasal half, and the former is nearly hemispherical in shape, whilst the latter is more conical. In the epibasal half the median and transverse walls arise consecutively, whilst in the hypobasal portion the first wall is formed in a plane parallel to that of the basal one. Subsequent divisions rapidly arise, and the parts of the young embryo become recognizable. There is a distinct foot, but it is not easy to distinguish its limits from the cells of the wall of the inner part of the archegonium, which divide freely and become densely filled with food materials (Fig. 37). The young sporophyte grows rapidly, and, as is so common with many aposporous ferns, the first-formed leaves show a marked tendency to produce prothallial outgrowths when the plants are cultivated under hurried conditions.

It is instructive to note that the prothallium of this variety behaves very differently from those of the common species of Hart's-tongue, when it assumes an apogamous development. In the first place, as described by Lang,[1] the embryo in the latter Fern arises from a 'cylindrical process,'

[1] Lang, Phil. Trans., vol. 190, p. 196.

and by the direct outgrowth of the sporophyte from the gametophyte. But in the variety *Drummondae* the sporophyte develops from the oosphere, and there is no cylindrical process. In the second place there is a very important difference between the behaviour of the nuclei of the prothallial cells in the two cases. In the aposporous prothallia of our variety there is neither nuclear migration nor fusion, but Lang describes and figures cells containing two nuclei as being situated 'near the border line between sporophytic and prothallus tissue.' We regard this as of paramount importance taken in connexion with the fact that whereas our prothallia were produced aposporously, those with which Lang was working had been grown from spores. It may therefore be taken as certain that whilst our prothallial nuclei, as regards the number of their chromosomes, are identical with the nuclei of the sporophyte, those of Lang's prothallium only possessed half the number characteristic of the sporophyte. Hence the nuclear migration (and probable fusion) in this case would fall exactly in a line with what we shall describe for *Lastrea pseudo-mas* vars. *polydactyla*, Wills and Dadds (see p. 176).

The results obtained from the study of this Fern may be summarized as follows:—

1. Aposporous development of the prothallia occurs on the margin of the leaf.

2. There is no reduction of the chromosomes on passing from the sporophyte to the gametophyte.

3. Whereas the ordinary Hart's-tongue Fern possesses 64 (premeiotic) and 32 (postmeiotic) chromosomes in the sporophyte and gametophyte respectively, the variety *crispum Drummondae* exhibits some amount of variation, and contains from 80 to 100 chromosomes in its nuclei.

4. There is no migration or fusion of nuclei in the cells of the prothallium of this variety.

5. The embryo arises apogamously from the unfertilized oosphere.

6. The oospheres may become enclosed each in a membrane before the archegonial neck opens.

5. *Lastrea pseudo-mas* var. *polydactyla*, Wills.

This Fern has long been recognized as one which only produces apogamous embryos on the prothallia, which are nevertheless raised from the ordinary spores. In 1903 we published a preliminary statement of the cytological observations we had made on the plant.[1] These observations not unnaturally were received with some degree of scepticism, but our further investigations have convinced us of the accuracy of our statements, and they have at the same time served, as we think, to invalidate

[1] Farmer, Moore and Digby, loc. cit.

some of the criticisms which, on somewhat *a priori* grounds, have been urged against them.

The prothallia on which our work has been almost exclusively based were given us by Dr. Lang, and they proved to be well-preserved for cytological investigation. We have not been successful in our efforts to raise material for ourselves, for spores sown in 1905 and 1906 for some reason failed to germinate[1].

As is well known, the prothallia of this variety of the Male Fern do not bear antheridia very freely, and the archegonia are wholly absent. In this respect it stands in sharp contrast with the variety *polydactyla* of Dadds, in which the sexual organs are produced abundantly, although no embryos are formed from the oospheres.

We believe that the fusion of the nuclei of adjacent vegetative cells replaces, in each of these two Ferns, the normal sexual act, and we will now give in some detail an account of the process as it occurs in *Lastrea pseudo-mas* var. *polydactyla*, Wills, the original plant of which was found many years ago growing wild in Devonshire.

The prothallia we used for this investigation were always mounted *whole* after staining, as with sections there is always a risk of misinterpreting the limits of particular cells, as well as the danger that the knife might have dislocated the nuclei from their original positions. The prothallia were stained in various ways, and on the whole we obtained the best results with Heidenhain's Iron-Haematoxylin.

The cells which are concerned in the migration are not limited to the thicker part of the prothallium. Those in the wings frequently afforded examples of the nuclear passage. It is, however, always in the younger regions of the plant that they occur, and it is useless to seek for them in the large full-grown cells at a distance behind the advancing margin.

The first sign of migration on the part of a nucleus is seen in a change of form (Figs. 45–47). It commonly assumes an elongated shape with the pointed end directed to the wall that it is about to pierce. The nucleus in what we may term the receptive cell is usually round in form, and appears to be quiescent. When the end of the invading nucleus comes in contact with the partition wall, it obviously produces some change, probably by means of a fermentative activity, and the apical part of the nucleus slips through the narrow orifice thus made. The anterior end of the nucleus contains a darkly staining substance, and it gradually squeezes itself through the aperture. The whole process is a very clear case of chemiotaxis, as is evidenced by the very definite way in which the penetrating nucleus makes its way towards the nucleus of the receptive cell, and it is perhaps also further indicated by the accumulation of stainable

[1] Since this was written we have succeeded in raising a number of prothallia showing the apogamous peg.

substance at its anterior end. After the nucleus has thus traversed the wall, the pore through which it has passed is sometimes difficult to identify, at other times, however, it remains as a well-marked hole. The nucleus then generally seeks the nucleus of the receptive cell, and the two lie closely appressed to each other, and finally they fuse (Figs. 48–51).

There are several variations that may be exhibited during the process. Sometimes the two nuclei do not immediately fuse, and we have seen many such instances of two nuclei lying in one cell, more or less near together, whilst there is invariably a non-nucleated cell immediately adjacent, from which one of the pair has come. At other times the fusion is very rapid, and both nuclei seem attracted to each other. Such an example is shown in Fig. 48, where the fusion is seen to take place almost simultaneously with immigration. A few cases which are perhaps to be regarded as 'abnormalities' occur, in which a nucleus will pass entirely through the adjacent cell, apparently attracted by the nucleus of one situated beyond it (Fig. 53), and, again, a few instances have been seen in which there are three nuclei in one cell (Fig. 52). But in every single case the origin of the invading nucleus was betrayed by the empty cell which it had abandoned. We have been able to trace the stages of fusion and division of the nuclei in question in a practically unbroken sequence.

The fusion assumes special importance when the chromosome contents of the nuclei come to be studied. We find in these prothallia that some of the dividing nuclei possess the same number of chromosomes as the dividing spore-mother-cells during meiosis, that is, they have the reduced number (Figs. 41, 42). In such nuclei we find 64 to 66 chromosomes. Of course there is a little variation in the actual numbers obtained, when dealing with chromosomes as numerous as these, but the latitude is not large. In other nuclei belonging to the cells of the same prothallium we find many more chromosomes. We are not able to state accurately what the actual numbers are, but they are certainly considerably over 100 (Fig. 43). We may fairly look on these as formed in nuclei which have duplicated the gametophytic (postmeiotic) number of chromosomes by fusion. The cells of the apogamously produced embryos have been studied, with the result that in every case we find these young sporophytes are characterized by nuclei possessing the double complement (premeiotic) number of chromosomes (Fig. 44). It thus appears to be clear that the nuclear cycle is essentially that of an ordinary sexual plant; that is, the sphorophytic premeiotic chromosomes are distributed at meiosis between the two pairs of spores which arise from the spore-mother-cell; from the spores arise the prothallial cell generations with the postmeiotic (reduced) chromosomes. But whereas in a normal life-history the premeiotic number is restored by the act of fusion of the egg and sperm nuclei, in this prothallium the same end is attained by the fusion of the nuclei of adjacent prothallial cells.

It may here be stated that we have investigated the prothallium of the common Male Fern, but have seen no cases of nuclear migration, nor have we been able to find any evidence of such migration in the case of the *aposporously* produced prothallia described below.

We find that the number of chromosomes at meiosis (e.g. the heterotype mitosis) in this Fern is somewhat less than that characteristic of the common Male Fern at the corresponding stage. For whereas in the latter it is about 72, in the variety *polydactyla* of Wills it is about 64–66 (Figs. 39, 40). The development of the embryo calls for no special remark. It is differentiated from the tissue of a prothallial excrescence, and we have nothing essential to add to the descriptions given by other authors. We have, however, given a few Figs. (54–56) to illustrate the process. It will be seen that it conforms very closely with the mode of development as it occurs in *Pteris cretica*,[1] and except for the presence of archegonia, with that of *Lastrea pseudo-mas* var. *polydactyla*, Dadds.

Comparing the size of the cells and nuclei of the prothallium with those of the typical form of the species, they are in both cases distinctly smaller, but there is not a consistent ratio between cell and nucleus. We find, as the result of a considerable series of comparisons of tracings made by the camera lucida of the cell network and nuclei of this variety and the type, that the average size of the cells is about 85 per cent., and of the nuclei about 60 per cent. of those of the common Male Fern. While we have taken all the care we could to make comparisons between prothallia that corresponded in age and development, it must be said that the measurements in any given single instance may show somewhat wide divergence from the mean or average. But nevertheless these numbers do convey very fairly the general impression gained as the result of comparing a considerable number of prothallia of the two Ferns.

6. *Lastrea pseudo-mas* var. *polydactyla*, Dadds.

The general habit of this Fern resembles that of the variety *polydactyla* of Wills, but it differs from the latter in many important points of development.

The spores are fertile, and large quantities of prothallia have been raised from them. The young plants are all produced apogamously, and those which we allowed to continue growing have retained the characters of the parent plant. We have studied the development of the archesporium in order to ascertain the number of chromosomes in the sporophyte. They are very numerous, and an accurate estimation, as in the preceding variety, could not be arrived at (Fig. 57). When the spore-mother-cells are formed the divisions are of the meiotic type, the first being typically heterotype and the second homotype. It is not necessary to enter into details of these

[1] Cf. De Bary, Bot. Zeit. 1878.

mitoses, but the number of the chromosomes was found to be greater than an investigation into the corresponding mitoses of the typical Male Fern had led us to expect. It is certainly not less than 90, and we are inclined to put it a little higher, 96 is probably nearer the actual figure (Fig. 58). This number is also met with in the prothallia (Fig. 59), although the chromosomes are more difficult to count in those cells than even in the spore-mother-cells.

The prothallia, as the investigations of others, especially of Lang, have shown, are normal in appearance. They bear antheridia and a few archegonia. The latter occur immediately behind the growing-point, and are situated upon a rather diffuse cushion. The antheridia are rather small. They arise as protuberances from the central part of the wall of a cell on the lower surface of the prothallial cells, and at first are easily confounded with young rhizoids. The antherozoids, as Dr. Lang remarks, are very active. The archegonia are peculiar in that they project considerably above the lower surface of the prothallium, and this is doubtless to be correlated with their mode of formation. The mother-cell divides at first by walls inclined to each other, and thus a young archegonium almost resembles a stumpy branch, and its elevated position is accounted for.[1]

The central, ventral, and neck canal-cells appear to be perfectly normal (Figs. 62, 63), and yet fertilization never takes place.

The embryo arises as a projection from the lower surface of the prothallium (Figs. 64, 65 *a*, *b*), and we confirm the statement of previous observers as to its proximity to an archegonium. We are inclined to regard the projection, which forms the first sign of the embryo formation, as the equivalent of an archegonium arrested and modified in the earliest stages.[2] Just as is the case with the archegonia, so also here, the first stage consists in the bulging out of one (or possibly more than one) superficial cell which becomes marked off from its fellows. This cell is, as has already been stated, most often situated close to an archegonium, and usually amongst several. It may be even derived from the peripheral cell of an archegonium itself, as has been stated by other investigators, but this has very seldom happened in our own cultures. The cell in question then divides by walls inclined to one another, and so a little patch of tissue, the cells of which are arrayed fan-wise in section, is formed. As is well known, the anterior portion of the projection develops into the stem-apex, whilst the root is formed by the projection backwards through the prothallium, and behind the venter of the archegonium, of a strand of cells. The protoplasmic contents of the constituent elements serve readily to distinguish them from the true prothallial cells around them. An apical cell is ultimately dif-

[1] Cf. the account given for *Doodia caudata* by Heim, Flora, Bd. 82.

[2] Lang has suggested a similar origin for the sporophytic 'buds' on the prothallia of *Aspidium angulare* var. *foliosum multifidum*, loc. cit., p. 205.

ferentiated on this posterior burrowing portion, and shortly afterwards the first cap-cell is cut off, and thus it is identified as the primary root. Tracheids and vascular strands are differentiated, and the embryo finally only differs from a normal one in the lack of a foot, and the position, external to the prothallium, of the stem-apex. It often happens that some of the prothallial cells situated near the anterior end of the embryo become divided so as apparently to form an additional conducting strand, connecting the embryo with the stores of prothallial nutriment. This tissue may perhaps be regarded as physiologically representing a somewhat imperfect foot, but formed by the prothallium instead of the embryo.

We have carefully examined a large number of prothallia for the nuclear migrations and fusions which are so constant and frequent a feature in the other variety of *polydactyla* (Wills). We have found them in relatively few cases (Fig. 61), and never in the wings of the prothallium. They have invariably occurred just behind the growing-point, and thus are confined to the region from which the young plantlet springs. We regard this fact as confirming the interpretation we have placed upon the process in the preceding variety, and it is of interest to find that whereas the migration and fusion of nuclei both occur in these two Ferns, the prothallia of which arise from spores, they are absent both from the common Male Fern, and also from all the other varieties which we have studied in which the prothallia were aposporously formed.

We have endeavoured to induce apospory in this variety as well as in the preceding one, but hitherto without result. The methods which have been so successful in the case of the other Ferns that form the subject of this paper have entirely failed here. We have examined the nuclei of the embryo (Fig. 60) in this plant, and find the number of chromosomes, as was to be expected, to be very great. Although on account of their crowded condition and great numbers we were unable to arrive at exact estimations, we see no reason to doubt that they are present in the typical premeiotic number.

The cells and nuclei of this variety, in spite of the numerous chromosomes, are smaller than are those of the type (cf. p. 186 below), and a similar character is shared by the antherozoids.

7. *Lastrea pseudo-mas* var. *cristata apospora*, Druery.

This Fern owes its origin to a prothallium which came up in a Wardian case in which *Trichomanes radicans* was growing. It seems perfectly clear from the account[1] given by its discoverer, Mr. Druery, that the prothallium in question must have grown from a stray spore which had found its way into the case. It was, in the first instance, referred to the well-known *Lastrea Filix-mas* var. *cristata*, in which apogamy had already been dis-

[1] Journ. Linn. Soc., vol. xxx.

covered by De Bary as long ago as 1878.[1] The general resemblances between it and De Bary's plant seemed to be fairly close, but our studies on these varieties of Ferns have convinced us that external features of similarity must be accepted only with considerable reserve, as affording indications of identity. The two forms of *polydactyla* varieties of the Male Fern, and those of *clarissima* in the case of the Lady Fern, prove that forms remarkably alike, but which have had a different origin, may be in essential characters very diverse. And this attitude of caution is justified in the present instance. For our plant is not only apogamous, but in a remarkable degree aposporous as well. Indeed, it is not known to produce spores at all. Whatever its real origin, it is at any rate now a plant essentially different from that originally known as *cristata*, and there is no sound reason for regarding the two as phylogenetically connected.

The plant is a small one, with leaves of delicate, somewhat pellucid texture. The vascular strands stop short of the ends of the pinnae, and the tracheids branch out in the form of a narrow brush. The leaf margins are abundantly provided with mucilage hairs, and very often the cells, especially at the ends of the final ramifications of the leaf, grow out in a tufted manner resembling the hymeneal layer of an agaric (Fig. 67).

On detaching the leaf and pegging it down on damp soil under glass, prothallia are produced in extraordinary abundance. They spring not only from the ends of the pinnules (Fig. 66), but also from superficial cells of the leaf (Fig. 68). These latter cells occur singly, and are not restricted to, though they are often more abundant in the vicinity of, the vascular strands. They are easily distinguished in prothallia that have been pegged down for a short time by the dense appearance of their nuclei and protoplasmic contents. The prothallia that arise from them are commonly irregular in shape, and sometimes assume a bulbous appearance. They did not, in our cultures, produce sexual organs or embryos.

The marginal and apical prothallia are much more regularly heart-shaped (Fig. 76), and in fact resemble ordinary prothallia of very delicate texture. Antheridia are produced freely, but archegonia have never been seen on them, nor do they appear to be formed at all. Most of the regular prothallia produce embryos, and occasional examples of tracheid formation have been encountered in prothallia that have not borne any embryo.

The appearance of the embryo is heralded by the formation of a very much localized hypertrophy situated just behind the growing-point (Figs. 74, 75). The cells divide freely, but the protuberance thus formed can hardly be described as a cylindrical process, such as is formed in *Lastrea ps.-m.* var. *polydactyla*, Wills. It is at best but a small excrescence standing out from the level of the prothallium, and tracheids very soon make their appearance within it. The formation of tracheids is not very definite at

[1] Bot. Zeit., 1878.

first, but ultimately a strand is formed which marks the primary vascular tissue of the young embryo. The root is formed endogenously, and bores out through the tissues in the posterior direction, whilst the first leaf and the apex of the stem are differentiated from the superficial cells of the excrescence. The embryo has thus a somewhat broad attachment to the prothallium (Fig. 76), but there is no structure that can be definitely recognized as a foot.

The chromosomes have been counted in dividing nuclei of the prothallium (Figs. 70, 71), in the antheridia, and in the embryo (Figs. 72, 73). The numbers obtained indicate, as in *Scolopendrium*, a certain degree of variation, but they quite preclude the occurrence of reduction in the sense of meiosis. For the prothallium, 60 is the probable number; the variation on either side, in nuclei favourable for counting the chromosomes, was very small. In the case of the embryo, on the other hand, whilst some nuclei showed 60, others exhibited a mean number of about 78.

It thus seems probable that the actual number of chromosomes is not quite constant even in the nuclei of the same individual. Possibly, as will also appear later, this variation in chromosomes is to be associated with other varying characters presented by these Ferns.

We searched a considerable number of prothallia to ascertain whether or not there is a migration of nuclei, such as occurs in the apogamous (but not aposporous) *Lastrea ps.-m.* var. *polydactyla*, but with a negative result. The importance of this becomes sufficiently obvious when it is remembered that there is no reduction in this plant, for with the omission of meiosis from the life-history there would appear to be no grounds for anticipating the intercalation of a process tending to duplicate the number of chromosomes. Indeed, with the cessation of the meiotic phase, it is difficult to understand how any such process could persist.

In comparing this Fern with the other varieties of *Lastrea*, it is impossible, especially if we fix our attention on the sporophyte, not to be struck by the relatively small number of chromosomes which the nuclei of its sporophyte contain. It is true that the other varieties diverge from the type in the matter of their complement of chromosomes, but their differences are insignificant as compared with that shown by the plant under consideration. If, however, we consider the gametophyte only, the difference is very slight, and the question naturally arises as to whether we are not, in this Fern, dealing with a case which is the correlative of those which we have hitherto considered; that is, whether, regarded from the standpoint of the chromosomes, the *gametophyte* character has not been impressed on the sporophyte, instead of the reverse process which we have encountered, for example, in the varieties of the Lady Fern. In the latter, we have every reason for believing that the gametophyte has arisen directly from the sporophyte, and owing to the absence of meiosis it has become endowed

with the premeiotic (unreduced) complement of chromosomes. In such a case there is no obstacle in the way of understanding that a sporophyte would naturally arise from the gametophyte by true apogamy, since the sporophytic number of chromosomes are already present. Such a view of the case would be further strengthened by a study of the *polydactyla* varieties of *Lastrea*. In them the prothallium is formed in the normal manner after meiosis, and the prothallial cells hence possess the postmeiotic (reduced) number of chromosomes. The embryo, it is true, is not the result of union of normal gametes, but nevertheless a nuclear fusion is concerned in its formation, and thus the full complement of sporophytic chromosomes is effected.

Of course the aposporous prothallium of the *Athyrium* type presents its own difficulties, but they may be surmounted if we admit that the special potentialities of the duplicate number of chromosomes may be reduced to latency. If, however, we admit, as indeed the facts seem to compel us to do, that not only is the formation of the gametophyte independent of reduction, but a sporophyte may arise directly from a gametophyte *which has itself arisen after meiosis*, i. e. without a restoration of the normal sporophytic duplication of chromosomes, we implicitly give up the position that alternation of generations is *necessarily* bound up with the periodic alternation in the number of the chromosomes. That is, we admit that there is no common *physiological* basis for the two processes.

We confess, however, that such a course seems to us to be the only one that is consistent with the facts. Of course it might be argued that, as in the variety *polydactyla* of Wills there is already evidence of a tendency towards reduction in number within the group of varieties, the var. *cristata apospora* only represents an extreme case. But apart from the improbability of such an explanation, having regard to the actual facts, we meet with great difficulties as soon as we refer to what is known about the origin of the variety. The nucleus of the spore which first found its way into the Wardian case must have only possessed the postmeiotic number of chromosomes, that is, only half the number of those of the sporophyte which produced it. Consequently up to this point, and on the formation of the prothallium by its germination, the character of each of the gametophytes of the three varieties of *Lastrea* must have resembled each other. The divergence began in the formation of the embryo. For whereas in the two *polydactyla* varieties the premeiotic number of the young sporophyte was provided for by a fusion of prothallial nuclei, this process was omitted in the variety *cristata apospora*, and thus no mechanism was utilized to ensure that doubling of the typical gametophytic number of chromosomes that so commonly is associated with the production of the sporophyte.

We shall defer the discussion as to the bearing of these conclusions on the question of alternation of generations to a later page.

Just as the chromosomes of this variety are fewer than those of the other varieties of *Lastrea* examined by us, so also the sizes of the cells, nuclei, and antherozoids are smaller than the corresponding elements in them. It is difficult in this, as in other cases, to give exact measurements, but taking the value of the respective structures in the typical *Lastrea pseudo-mas* as 100, a series of careful comparisons gives for this variety the following results :—

Prothallial cells, 60 ; nuclei of prothallial cells, 75 ; antherozoids, 90.

General Discussion.

A consideration of the structure of the so-called varieties of *Lastrea* and of *Athyrium* which have been described in this paper serves to show that with the variation in form other important characters are associated. These characters not only concern the peculiarities connected with the particular form of apospory or apogamy that may be exhibited by an individual variety, but they extend to minute details of cell structure. Thus the 'varieties' of *Athyrium* show, though in various degrees, a constant difference in the nucleoli of their prothallia as compared with those of the type. They all possess numerous chromatin nucleoli, and these assume at times very remarkable shapes. Moreover, there is in this respect a fairly regular amount of deviation from the type which marks the individual variety and distinguishes it from the others. Whereas the prothallial nuclei of the ordinary Lady Fern, *Athyrium Filix-foemina*, possesses commonly one or sometimes two nucleoli, those of the variety *clarissima*, Bolton, show a number of these bodies.

The corresponding nuclei of var. *clarissima*, Jones, possess the same peculiar nucleoli, consisting chiefly of chromatin, but they are more abundant and often assume very curious forms, at times recalling those of heterotype chromosomes, with which, however, they have really nothing to do ; they are simply nucleoli of very unusual form, and when mitosis takes place the chromatin from them passes out into the linin threadwork of the nucleus. The nucleoli of the variety *unco-glomeratum*, again, are also numerous, but they lack the unusual appearance presented by those of the former species. Another series of constant differences, which are probably of greater interest, lie in the size of the cells and nuclei of the different varieties. In *Lastrea* these differences are somewhat irregular, but in *Athyrium* we find them arrayed in a series in an almost regular manner. We will consider the case of *Athyrium* first, and it will conduce to clearness if we record the gradations as we have observed them in a tabular form, premissing, however, that the numbers given are not intended to express more than the average results obtained after the study of a considerable mass of material. They may, however, be taken as expressing the proportionate size as given by a large number of camera lucida drawings made to scale from the cells, &c., of

organs as nearly corresponding in their stages of development as we could get. We have reduced the value of the different cells and nuclei so as to express them in terms of the standard (100) given in the case of *Athyrium Filix-foemina*, which, for purposes of this comparison, we have selected as the type of the *Athyrium* series. We have also added the number of chromosomes.

	Athyrium Filix-foemina (type).	*Ath. F.-f.* var. *clarissima*, Bolton.	*Ath. F.-f.* var. *clarissima*, Jones.	*Ath. F.-f.* var. *unco-glomeratum*, Stansfield.
Young prothallial cells (near growing-point)	100	135	180	250
Old prothallial cells (in the wings)	100	140	170	300
Young prothallial nuclei	100	140	190	250
Old „ „	100	160	220	280
Epidermal cells of leaf of young sporophyte	100	110	180	200
Antherozoids	100	135	160	250
Number of chromosomes (actual number)	76–80 (38–40 in the prothallium)	84	90	100

It will be seen that there is a fairly constant difference characteristic of each so-called variety. It should be stated that of course the cells of the young parts of the prothallium are much smaller than they ultimately become, and that the nuclei in the older cells are likewise smaller than those of the cells at the growing-point. But this age-difference is tolerably uniform throughout the different varieties, as is indicated by the figures in the foregoing table.

In the young cells we found the ratio $\left\{\frac{\text{size of nucleus}}{\text{size of cell}}\right.$ to approximate very closely to 0·32, but at a short distance behind the growing-point, owing to the relatively great enlargement of the cell, it diminished to 0·07 and less.[1] It must be clearly stated that these relations only have to do with size, the chemical characters, such as nuclein content, having been left out of consideration. But taking the cell and nuclear size as the criterion, it will be readily seen that the so-called 'varieties' are severally removed from the type in the order in which they are placed in the table. It has been seen that the nucleolar characters differentiate them in the same way, and the size of the antherozoids and the number of the chromosomes both point in this same direction. Furthermore, the facts in connexion with apogamy are also in harmony with the other features. *Clarissima*, Bolton,

[1] Cf. Strasburger, Ueb. d. Wirkungssphäre d. Kerne u. d. Zellgrösse, Hist. Beitr., v. Schwartz, Beitr. z. Entw. d. pflanzl. Zellkernes nach d. Theilung, Cohn's Beitr. z. Biol. d. Pflanzen, Bd. 4. Gerassimow, Ueb. d. Grösse d. Zellkernes, Beitr. z. Bot. Centralbl., Bd. 18; also, Die Abhäng. d. Grösse d. Zelle v. d. Menge d. Kernmasse, Zeitschr. f. allg. Physiol., Bd. 1.

with its embryo arising from the oosphere, is plainly nearer the type than is either *clarissima*, Jones, or *unco-glomeratum*, Stansfield, in which it arises from the vegetative parts of the prothallium.

It is furthermore of special interest to find that the same sequence is shown by a comparison between the cells of the first leaves of the sporophyte generation, taking the epidermal cells as the basis of measurement. We selected these because there was less likelihood of the introduction of error, since the uninjured leaf can be used. Sections introduced so many complications as to render any estimations based on them almost valueless.

It would be doubtless going too far to suggest that these varieties of the Lady Fern should be regarded as of specific rank. The fact that the offspring are to some extent variable and unstable would alone render such a course undesirable. But they may perhaps be justly considered as 'mutations' which possess the faculty of continuing to vary, and even apparently of reverting to the parent form. It remains, however, to be seen whether these apparent reversions are really to be so regarded, and an attitude of caution respecting them is desirable, at any rate for the present. We propose to investigate the progeny of the forms here studied in the immediate future, in the hope of deciding some of the questions here suggested.

The forms of *Lastrea* are less regular in their divergence from the parent form and from each other than are those of *Athyrium*. In one sense, however, the trend of variation is in a direction opposite to that prevailing in the varieties of the latter genus, at least so far as we know them at present. For whereas in *Athyrium* the type is surpassed by its varieties in cell-size, &c., in the three varieties of *Lastrea* the reverse is the case. Of course a more extensive study of the varieties of each Fern may show that this difference is merely accidental, and that each type really stands more in the mean than at one end of the variations.

Treating *Lastrea pseudo-mas*, and its three varieties studied by us, in the same manner as the former genus, the subjoined table will summarize the general nature of the differences.

	Lastrea pscudo-mas.	*Lastrea ps.-m.* var. *polydactyla*, Wills.	*Lastrea ps.-m.* var. *polydactyla*, Dadds.	*Lastrea ps.-m. cristata apospora.*
Prothallial cells of average size (near growing-point)	100	85	70	60
Nuclei of ditto	100	60	70	75
Epidermal cells of average size (sporophyte)	100	90	100	70
Antherozoids	100	?	85	90
Chromosomes:—				
gametophyte	72	66	90	60
sporophyte	144	132	130	66? 60 & 78

The chief points of interest brought out by the foregoing table are, firstly, the small size of the nuclei as compared with cells in *polydactyla*, Wills, and the reversed relation in the case of *cristata*; secondly, the fact that whilst the cells of *polydactyla*, Wills, are larger than those of the corresponding variety of Dadds, the chromosomes of the latter are far more numerous. As regards the antherozoids, it was not easy to arrive at exact estimations, but we incline to place those of *cristata* as slightly larger than those of *polydactyla*, Dadds; we were unable to get satisfactory measurements of the antherozoids of the other variety. The measurements of the cells of the leaves[1] did not, as will be seen, give concordant results.

We may, for the moment, omit from our consideration the chromosome characters as presented by *cristata*, since they are affected by conditions different from those that concern the two varieties of *polydactyla*. The general result of a comparison of these forms shows that in internal, just as in larger external characters, divergence from the type may affect individual features independently, and this is strikingly shown by the smaller number of chromosomes possessed by the cells of *polydactyla*, Wills, and the larger number exhibited by the variety of Dadds, when both are compared with the type. This independence, as has been seen, extends to other characters within this group, and its existence serves to correct any impression of rigid correlation of differences with any one variant (e. g. chromosome number) such as a study of the forms of *Athyrium Filix-foemina* alone might perhaps have suggested.

It seems not unlikely that comparative investigations on somewhat similar lines might usefully be extended to groups of allied species of other alliances, and it would be especially interesting to obtain this information in respect of those congeries of forms known as sub-species, mutations, and the like, in the case of flowering plants.

It is now necessary to consider the question of the chromosomes more in detail.

It has already been stated, in dealing with the individual forms, that the actual numbers obtained in each case are to be taken only as averages, but that the countings themselves yielded a certain divergence from each other round the mean. We are disposed to regard this divergence as not entirely due to errors in estimation, but as in part, at any rate, representing a real difference. It has previously been shown[2] that differences occur in plants which only contain so few chromosomes as to make it impossible that the differences in the numbers estimated could be attributed

[1] In the intercellular spaces of the leaves of each variety the well-known glands are of frequent occurrence.

[2] Farmer and Reeves, On the Occurrence of Centrospheres in *Pellia epiphylla*, Nees. Annals of Bot., vol. viii. Farmer and Shove, On the Structure and Development of the Somatic and Heterotype Chromosomes of *Tradescantia virginica*. Quart. Journ. Micr. Sci., vol. xlviii.

to errors of counting. And although in the plants now under discussion it is impossible to speak with absolute certainty as to the degree of error that is unavoidably present, such a case as that of *Lastrea cristata apospora* is well worthy of attention. Since the estimation of the chromosomes in the prothallium, with two exceptions, all fell between 59 and 64, with a great preponderance in favour of the former, we think we are probably correct in taking 60 as fairly representing the chromosomes in the prothallium. But with the embryo it is different. We find our numbers fall into two discontinuous groups, one ranged closely about 60 (59–64) the other approximating to 78 (77–80). Similarly, in respect of *Scolopendrium vulgare* var. *crispum Drummondae*, whilst the prothallial countings gave 70–75 as an average number, it was clear that the embryo contained more, ranging perhaps from 95–105, or say 100, as a mean. These indications of individual differences are of interest when taken in connexion with the varietal differences. For, along with much evidence—which conveying from many sources seems to testify to the permanence of the chromosomes—we meet notwithstanding with positive cases of chromosome fluctuation that might seem to negative any value being attached to number.

Even in other plants and animals, in which the numbers are low, we find differences in closely allied species which are difficult to account for, although they prove that the fractionation of the linin that bears the chromatin is subject to change, introduced either through rearrangement of the primoridia, or owing to the limits between individual chromosomes losing their original precision. And if, as we have strong grounds for believing, the chromosomes are intimately associated with hereditary qualities of the organism, it is evident that a disturbance of the chromosome structure would, *ceteris paribus*, be likely to be associated with variation. But it is evident that one must keep distinct two kinds of chromosomic differences. In the ordinary somatic mitoses the entire nuclear linin and chromatin is equally halved between the two daughter nuclei, and for this purpose it is difficult to see how the mere chance of any temporary end-to-end adherence—or even an increased fractionation—of the individual chromosomes could affect the final result. But in meiosis, where there is a segregation and ultimately a distribution of units contributed by the paternal and maternal ancestors respectively, it is readily comprehensible that any such destruction of the individuality of the chromosomes might find expression in the production of sports, or unlooked-for variations.

Regarding the instances of apogamy and apospory which have been described in this paper from the point of view of their nuclear history, it is clear that there are two main groups into which they severally fall. Firstly, we have forms in which fertile spores are produced in connexion with the ordinary process of meiosis. To this category the two varieties *polydactyla* of the common Male Fern belong. Secondly, there are those

plants in which spores (and the meiotic phase) are cut out of the life-cycle, and the gametophytes arise directly as outgrowths from some part of this sporophyte—whether from barren sporangia or from the tissues of the leaves, and retain the full sporophytic (premeiotic) complement of chromosomes. The varieties *clarissima* of the Lady Fern may be cited as examples amongst the Ferns. Perhaps a third group should be added in which there is reason to think (e. g. *Lastrea pseudo-mas* var. *cristata apospora*) that the sporophyte only retains the original gametophytic chromosomes, but as in this form apospory and apogamy regularly follow each other, and the gametophyte *now* resembles the sporophyte in its chromosome contents, it is better to avoid increasing the complexity of classificatory systems by inventing a special class for such cases.

Most people would probably agree with us in regarding the type of apogamy, as illustrated by the *polydactyla* varieties, as simpler and less modified than that which involves apospory as well. The prothallia of many Ferns belonging to the former series are apparently normal, and they are certainly so during their earlier life. Thus we meet with facultative apogamy in the latter, which passes into obligate apogamy in those Ferns which have lost the power of producing archegonia. In the cases examined, the method of doubling the chromosomes of the gametophyte by ordinary fertilization is replaced by the fusion of nuclei belonging to prothallial cells. There is very strong evidence to show that this latter process obtains in prothallia that have arisen from fertile spores, but which by cultural conditions have been prevented from forming sporophytes other than in an apogamous manner, for Lang[1] observed and figured binucleated cells in his prothallia of *Scolopendrium vulgare*, very similar to those we have found in *Lastrea*. On the other hand, when the prothallium arises aposporously, as in the varieties of *Athyrium*, in *Lastrea pseudo-mas* var. *cristata apospora*, and in *Scolopendrium vulgare* var. *crispum Drummondae*, no such nuclear fusion has been detected. The cases are closely paralleled by what is known to occur when meiosis is omitted elsewhere. Thus Overton[2] found that in *Thalictrum purpurascens* the embryo could arise with or without fertilization. He adduced convincing evidence to prove that in the latter case meiosis had been suppressed, and that the oosphere itself possessed the full premeiotic complement of chromosomes. Such a case, which is not one of parthenogenesis, but a true case of apogamy, is very similar to that of *Athyrium Filix-foemina* var. *clarissima*, Bolton, and the *Drummondae* variety of the Hart's-tongue. The chief difference is that in the *Athyrium* the gametophyte springs from premeiotic sporangial

[1] Lang, loc. cit.

[2] Overton, J. B., Ueber Parthenogenesis bei *Thalictrum purpurascens*. Ber. d. Deutschen Bot. Gesellsch., Bd. xxii.

cells, instead of from spores which had not undergone meiosis, and in the Hart's-tongue the gametophyte arises still more directly from the sporophyte (leaves). Rosenberg has recently shown in *Hieracium excellens* and *H. flagellare* [1] that one and the same plant may show differences in the seat of origin of its gametophyte. Some of his plants were normal, and produced a postmeiotic gametophyte in an ordinary embryo-sac or spore. Others formed a macrospore but omitted meiosis. Others, again, replaced the developing, but finally aborting, spores by a cell originating from the nucellar tissue, i.e. from an extra-archesporial cell, just as in the varieties *clarissima* of *Athyrium*.

In the two last cases the sporophyte arose apogamously from an oosphere already provided with the premeiotic number of chromosomes. Juel [2] has also shown in *Taraxacum* that whilst meiosis occurs in connexion with the formation of the microspores, it is absent from the series leading to the formation of the macrospore and oosphere. Furthermore, in connexion with the mitosis leading to the formation of the macrospore or embryo-sac, he makes the interesting observation that the earlier stages of nuclear division indicate the onset of the meiotic phase. The appearance is, however, transitory, and the mitosis soon assumes the character of an ordinary premeiotic division. A somewhat similar state of things was also described by Strasburger [3] for the *Eualchemillas*, in which Murbeck [4] had also discovered that apogamy occurred.

A fusion of the nuclei of the vegetative cells closely similar to, but indeed not physiologically identical with, what happens in the *polydactyla* varieties of the Male Fern, has been shown by V. H. Blackman [5] to take place in the Uredineae, and perhaps in a more disguised form in the Ascomycetes. Possibly the cases of anomalous conjugation of cells belonging to the same filament in *Spirogyra* might be looked upon as examples of an analogous nature.

It would thus seem to be desirable to restrict the term apogamy, or *Eu-apogamy*, to those examples in which, as in *Athyrium*, *Eualchemilla*, &c., there is no pretence of, as there is no obvious need for, fertilization of any kind; and we would suggest the term *Pseud-apogamy* to cover those instances in which the sexual fusion of gametes is replaced by a fusion of ordinary gametophytic nuclei which, morphologically, are not sexually differentiated. This would include the process as it occurs in the Uredineae

[1] Rosenberg, O., Ueber d. Embryobildung in d. Gattung *Hieracium*. Ber. d. Deutschen Bot. Gesellsch., Bd. xxiv.

[2] Juel, H. O., Die Tetradentheilungen bei *Taraxacum* u. anderen Cichorieen, Kongl. Svenska Vet. Ak. Handlingar, Bd. 39.

[3] Strasburger. D., Die Apogamie der *Eualchimillen*. Pringsh. Jahrb. f. wiss. Bot., Bd. xli.

[4] Murbeck, Sv., Parthenogenetische Embryobildung in der Gattung *Alchemilla*. Lunds Universitets Årsskrift, Bd. 36.

[5] Blackman, V. H., On the Fertilization, Alternation of Generations, and General Cytology of the Uredineae. Ann. Bot., vol. xviii.

and probably the Ascomycetes, and also the growing number of instances of apogamy in prothallia induced by cultural conditions, as well as such cases of obligate apogamy as that of the *polydactyla* varieties of the Male Fern.

As a sub-class of the eu-apogamous developments it might be convenient to distinguish as *parthenapogamous* those forms in which the sporophyte originates from a (premeiotic) oosphere. In this way it will be possible to avoid confusion with parthenogenesis. It is true that at present we do not know any well-founded cases of parthenogenesis, but such may nevertheless be discovered. It would of course involve the origin of the sporophyte directly from the postmeiotic unfertilized oosphere, which would then rise to give a sporophyte with a similar nuclear character. The known examples of parthenogenetic Echinoderm larvae artificially produced indicate the desirability of keeping an open mind as regards the possible occurrence of parthenogenesis, and it may be pointed out that if *Lastrea pseudo-mas* var. *cristata apospora*, when it first developed from a spore, had possessed archegonia it might have been quite possible for a parthenogenetic production of the first sporophyte to have taken place.

When apospory, and with it the omission of meiosis, has occurred in the life-cycle, then an apogamous development seems the only way of continuing the life-history; and, *vice versa*, it is evident that apospory might also be correlated with parthenogenesis.

We propose to present in the following table a suggestion for the classification of the various apogamous and aposporous types, but in framing such a classification we have sought to avoid making it too cumbrous by too minute subdivision. We are well aware, however, that no single form of classification is likely to meet with universal acceptance, and we only offer this as one which we have found useful ourselves.

A. After Meiosis.

1. *Normal fertilization*:
   Example: the ordinary life-history of archegoniate plants.
2. *Pseudapogamy* (facultative or obligate):
   Examples: *Lastrea pseudo-mas* vars. *polydactyla*; Uredineae (Blackman).
3. *Euapogamy*:
   Example: *Lastrea pseudo-mas* var. *cristata apospora* at its first origin.
4. *Parthenogenesis*:
   No case at present definitely known.

B. MEIOSIS ABSENT, apogamy obligate.

1. *Parthenapogamy*, sporophyte originates from oosphere—

(α) After formation of spores:

Examples: *Thalictrum purpurascens* (Juel), *Eualchemilla sp.* (Murbeck, Strasburger), *Hieracium excellens* (type 2 of Rosenberg), *Antennaria alpina* (Juel).

(β) With apospory:

Examples: *Athyrium Filix-foemina* var. *clarissima*, Bolton, *Scolopendrium vulgare* var. *crispum Drummondae*, *Hieracium excellens* (type 3 of Rosenberg).

2. *Euapogamy*, sporophyte originates from gametophytic tissues—

(α) After formation of spores:

Example: possibly *Lastrea pseudo-mas* var. *cristata apospora* at its first origin.

(β) With apospory:

Example: *Athyrium Filix-foemina* var. *clarissima*, Jones; *Lastrea pseudo-mas* var. *cristata apospora*.

Of course any classification is likely to contain anomalies, and it may be objected that it is unnatural to make the presence or absence of spores a means of subdivision. It is true that it is not perhaps very logical, but it is a convenient character, especially in connexion with the Phanerogams, although there is in them one irregular feature that demands a passing comment. The limits between the spore and the spore-mother-cell are apt to disappear, and thus it might be argued that, strictly speaking, many forms are really aposporous which are not usually reckoned as such. For example, when the spore-mother-cell at once becomes the cell in which the gametophyte is produced, as in *Lilium*, one might—though not very usefully—maintain that the spore stage properly so called had been cut out of the life-history.

On the whole we have preferred to regard all the single cells in which the phanerogamic gametophyte is produced as spores, whether they go through the tetrad division or not, if there seems to be sufficient reason to refer them to an archesporial origin. In Rosenberg's type 3 of *Hieracium excellens* and *H. flagellare* the single cell in which the gametophyte is produced is clearly not referable to such a source, and it is therefore classed with the aposporous forms. This distinction is the more necessary because of course the gametophyte of the Phanerogams is always intracellular, but it is theoretically quite possible for the enclosing cell to belong even to extra-sporangial tissue, although no instance of the kind, so far as we are aware, has been as yet discovered.

Amongst the Ferns, we are not at present acquainted with an example in which spores are produced in the absence of meiosis, but their relatively common occurrence in the ovules of Phanerogams renders it not im-

probable that such an instance may turn up amongst these plants. If so, the spores on germination might give rise to prothallia consisting of premeiotic cells, and it would then be fairly safe to assume that any sporophytes formed on them would likewise be apogamously produced.

We have purposely refrained from discussing those cases of apogamy in which nothing is known as to cytological details, as, for example, *Balanophora*, *Ficus*, *Gnetum*, *Allium*, and many others. For the assigning of these to their proper places can only be carried out when they have been reinvestigated from the point of view of their nuclear structure.

Alternation of Generations.

It is perhaps impossible, in dealing with the cytological aspect of apospory and apogamy, to avoid reference to questions involving views as to the nature of alternation of generations in the archegoniate plants. Within recent years a certain amount of cytological evidence has been woven into the fabric of theory, with the result that a conviction seems to have been formed in the minds of some botanists that the antithetic theory is in some way bound up with the periodic change in the number of the chromosomes. Consequently the facts that have now come to light, proving that gametophyte may rise directly from sporophyte, and sporophyte from gametophyte, without any such change on the part of the chromosomes, have been looked on as sapping the foundations on which the antithetic theory rests; and, *pro tanto*, as supporting the views of those who advocate the theory of homologous alternation. As a matter of fact, it appears to us that they tend neither to destroy the one nor uphold the other.

The periodic change in the number of chromosomes is primarily related to sexual fusion. Sexuality is a condition of meiosis, and meiosis in its turn renders the continuance of the sexual act a possibility; at least this is a fair statement of the matter so far as our present knowledge is concerned. Just as sexuality is the common property of all the animals and plants alike (with sundry exceptions that in no way invalidate the general position), so also is meiosis. And the details of the mitoses included in the meiotic phase are extraordinarily similar in the two kingdoms.[1] Thus one would hardly be guilty of exaggeration in stating that, just as the fundamental importance of sexuality is rightly gauged from its almost universal occurrence, so also is that of the intimately related meiotic phase, regularly intercalated as it is in the life-cycle of every sexually reproducing animal and plant.

[1] Farmer and Moore, On the Essential Similarities existing between Heterotype Nuclear Divisions in Animals and Plants. Anat. Anzeiger, 1895.

In fact, these two processes—sexuality and meiosis—are knit together by the closest physiological ties. They stand each to each in a very definite relationship of a causal nature. But there exist no *a priori* grounds for assuming any such necessary connexion between either of them and any other features or phases in the life-history, however important these may be in themselves. The matter becomes one for independent inquiry, in the midst of which it should not be forgotten that *post hoc* is not the same thing as *propter hoc*. Meiosis may well be found to concur with certain events or stages in particular life-histories without any assumption as to the existence of any essential or necessary relation being in any way justified. The fact that meiosis long anteceded the appearance of the Archegoniatae might indicate that the hypothesis as to its necessary relation with individual parts of the life-history in these plants requires to be tested by a comparison with other groups before it can be admitted. But such a comparison with forms outside the archegoniate series at once shows that the fundamental assumption underlying the hypothesis is not of general application, nor indeed is it universally true even within the series itself. Meiosis occurs in all the groups (except perhaps the very lowest, in which sexuality is not known), but it does not recur at corresponding periods in all alike. Thus, to take the first example of *Fucus*, the meiotic phase involves the two first nuclear divisions in the oogonium, whilst the last is a post-meiotic division, and results in the formation of the (potentially eight) oospheres. But no one probably would seriously attempt to homologize the product of the first two divisions of the *Fucus* oogonium with the spores of a Fern, and to conclude that the final division in the oogonium morphologically represented the prothallium reduced to a mere oosphere. The Dictyotaceae present some features of difficulty, but these are more apparent, perhaps, than real. Williams has shown that the plants bearing sexual organs are made up of postmeiotic cells, the plants that issue from the zygote, on the other hand, are premeiotic, meiosis regularly occurring during the formation of the tetraspores. *Dictyota* is far removed from the Archegoniatae, and it is not sound morphology to attempt to drag into homologous series groups which have had a different phylogenetic development. At present, moreover, we are not fully in possession of the facts respecting *Dictyota*, but even if it should ultimately turn out that there is a regular alternation between a tetrasporic and a sexual generation, the question as to the nature of the alternation itself is one which will have to be settled on its own merits, and not by an appeal to a widely diverse group such as the Archegoniatae. What we shall require to know is, whether there is any evidence to show that within that algal group there has been built up from the *zygote* a cell-colony which, from a condition of a parasitic embryo, has achieved independence as an asexual spore-bearing individual. It is, on the other hand, still quite

conceivable, though as we think improbable, that *Dictyota* may afford a true example of homologous alternation; this would not, however, affect the question of the origin of the sporophyte in the Archegoniatae.[1]

In the Desmids and the Conjugatae, as Klebahn and others have shown, there exist tolerably conclusive reasons for believing that meiosis occurs immediately on the germination of the zygote, and that consequently the whole plant belongs to the postmeiotic stage of the cellular cycle, thus forming the exact antithesis of what occurs in *Fucus*.

Once more, Allen has shown in *Coleochaete* that meiosis is bound up with the first two divisions, that is with the germination, of the zygospore. Consequently, the cells of the 'sporophyte' organism lying within the oogonium belong to the postmeiotic cell generations. But the ordinary Coleochaete plant only arises from the zoospores which are produced by this 'sporophyte.' But in spite of this early onset of meiosis probably few would refuse to admit the claim of the 'sporophyte' of this plant to be regarded as an intercalated cell series. The Coleochaete *plant* is not formed till the emitted zoospores give rise to it, and the cell divisions which intervene between zygosis and their formation can hardly be looked on as other than a new and intercalated development. It is not very likely that any one would maintain that the sporophyte was the modified representative of a normal *Coleochaete* plant, whereas it is easy to see how it might have arisen on the lines of *Oedogonium* or *Sphaeroplea*.

But the fact that in *Coleochaete* the 'sporophyte,' after it has passed the first stages of germination, should consist of postmeiotic cells affords an interesting contrast with what happens in the Archegoniatae. In both cases the spore (or zoospore) marks the natural transition to the gametophyte, and since we find that meiosis is not of necessity bound up with uniform stages in the life-history of all organisms, there appears to be little justification for a refusal to admit the claims of *Coleochaete* to be regarded as a type of alternation on all fours with, though forming a variant of, that exhibited by the Archegoniatae. We frankly confess that such a comparison appears to us more probable than a view which would relegate the postmeiotic cell-generations to a position analogous to that of the protonema of a Moss.

Finally, the flowering plants themselves afford perhaps the best proof of the statement that whilst meiosis is of fundamental physiological importance it is nevertheless not bound up with definite morphological limitations. In the majority of cases, it is true, it does coincide with the onset of particular morphological changes, but it is really independent

[1] Recent investigations have shown that in the relation of the incidence of the meiotic phase the Florideae, in which there are cogent reasons for admitting the existence of a true alternation of generations, resemble the lower members of the archegoniate series. Cf. Yamanouchi, The Life-history of *Polysiphonia*, Bot. Gazette, vol. xlii.

of them. In the spore-mother-cells as they are formed in the ovule or macrosporangium, provided that these divide into four cells, as phylogenetically speaking they ought to do, then the meiotic phase coincides with this division into four potential macrospores. But if the now useless stages are cut out, we find meiosis shifted onwards in the ontogeny until, as *Lilium candidum* for example, it may even coincide with the first two divisions of the germinating macrospore, that is to say, it has been shifted on to the developing gametophyte. Inasmuch then as it appears that no *essential* relationship subsists between the onset of meiosis and any particular stage in the life-history, the question at issue between the respective champions of the antithetic and homologous theories, in so far as cytological evidence is concerned, remains very much where it was before. But notwithstanding this, it seems to us that the attention which has become necessarily concentrated on these questions has indicated wider points of view, and at least has served to render the real issues somewhat clearer.

It hardly needs pointing out that the views as to alternation are in no way affected by the circumstance that in so many of the higher plants we meet with exceptions in which the ordinary course of events is not adhered to. Thus cells that should have continued to perpetuate their like until the final appearance of asexual[1] reproduction may depart from this prescribed course, and so, as it were prematurely, may give rise to the next (alternate) generation. Amongst the lowest plants such an instance would be afforded by a zygote of *Oedogonium*, which should at once grow out into an Oedogonial filament, with the omission to produce the four zoospores. This might conceivably represent a primitive case of apospory, but if the filament thus arising were to reproduce sexually one might pretty safely assume that the meiotic phase had not been suppressed, as in the higher plants where sexuality correspondingly fails, but that it probably occurred on germination, although unaccompanied by the usual formation of asexual zoospores.

The circumstance should not be lost sight of that we are profoundly ignorant of the causes that guide the stages of ontogeny, and it seems now certain that any cell the nucleus of which is provided with the requisite chromosomes, whether these are in single or duplicate number, is at least potentially endowed with the capacity of forming the starting-point of the entire life-history, in so far as the grosser morphological characters are concerned. Thus tissues with premeiotic nuclei can yet become transmuted into the generation normally characterized by post-

[1] We use this word to denote those cells which normally terminate the one and form the starting-point for the next (sexual) generation. We prefer, according to older usage, to retain the spore as the natural boundary between the two generations rather than to adopt the spore-mother-cell as such, according to the view suggested by Strasburger in 1894 and supported by some distinguished American botanists.

meiotic nuclei, and the case of *Lastrea pseudo-mas* var. *cristata apospora* shows that the converse is also possible.

The general conclusion to be drawn from this somewhat lengthy discussion, on the relation between the periodic reduction in the number of the chromosomes and the alternation of generations, is that no necessary correlation exists between the two phenomena, and therefore the problem of alternation and its nature must be settled by an appeal to evidence other than that derived from the facts of meiosis. That alternation is *normally associated* with meiosis on the one hand and with zygosis on the other no one will dispute so far as the Archegoniatae are concerned. But in the absence of all proof of the existence of a necessary and causal connexion, or rather in the face of direct evidence to the contrary, it is scarcely to be wondered at that the presumed correlation should often be broken as ontogeny becomes more complex; and thus the way is paved for the appearance of exceptions which, so long as they are of rare occurrence, are often regarded as monstrosities or abnormalities; but when they become common they are rather to be looked upon as proofs of current physiological instability than as keys for the immediate solution of problems in morphology or phylogeny.

EXPLANATIONS OF FIGURES IN PLATES XVI–XX.

Illustrating Professor Farmer and Miss Digby's paper on Apospory and Apogamy in Ferns.

[The nuclei have all been drawn with the camera to the same magnification (× 1500) 3 mm. Hom. Imm. Zeiss. Oc. 18, except Fig. 32, which was drawn under 2 mm. Hom. Imm. Zeiss. Oc. 18 (× 2250).]

PLATE XVI.

Athyrium Filix-foemina var. *clarissima.*

Fig. 1. Sporangium (young) before the archesporium is delimited.

Fig. 2. Sporangium with apical prothallial growth (*p*).

Fig. 3. Prothallial growth, 4-celled.

Fig. 4. Abortive sporangium with three prothallial outgrowths.

Fig. 5. Multicellular prothallium from apex of the abortive sporangium; the archesporial tissue (*a*) can be distinguished.

Fig. 6. Abortive sporangium with four prothallial outgrowths.

Fig. 7. Abortive sporangium with older prothallium.

Fig. 8. First prothallial cell in division, nucleus in equatorial plate stage. The cells below the one in division belong to the sporangium.

Fig. 9. Nucleus from wall of young sporangium in equatorial plate stage, for comparison with the preceding figure.

Figs. 10, 11, 12. Nuclei of young prothallial cells. *nu*, nucleoli. × 1500.

Fig. 13. Prothallial nucleus in early prophase of mitosis. × 1500.

PLATE XVII.

Fig. 14. Prothallial cell with nucleus in equatorial plate stage. × 1500.

Fig. 15. Nucleus of the antheridial cell in equatorial plate stage; the daughter-cells will give rise to the spermatocytes. × 1500.

Fig. 16. Spermatocyte with antherozoid differentiating. × 1500.

Fig. 17. 'Bulbous' prothallium on frond. Three reduced prothallia are also shown. From a cleared preparation. *f*, surface of leaf; *Ar*, archegonium; *s*, abortive sporangium with prothallial outgrowth.

Fig. 18. 'Bulbous' prothallium bearing embryo, from a preparation mounted entire. *f*, the surface of the leaf.

Fig. 19. 'Bulbous' prothallium with earliest rudiment of sporophytic tissue (*sp*) (longitudinal section).

Fig. 20. 'Bulbous' prothallium (section) with young embryo.

Fig. 21. *a*, *b*, *c*, *d*, four serial sections through a bulbous prothallium bearing an embryo.

Fig. 22. Section through older embryo. *R*, the root.

Figs. 23, 24. Nuclear division in the cells of young embryo.

Athyrium Filix-fœmina var. *clarissima*, Bolton. × 1500.

Fig. 25. Nucleus of prothallial cell. × 1500.

Fig. 26. Nucleus of prothallial cell in mitosis. × 1500.

Fig. 27. Nuclear division in embryo. × 1500.

PLATE XVIII.

Fig. 28. Oosphere (contracted) in the venter of the archegonium. *n*, neck of archegonium; *a*, antherozoids.

Fig. 29. Embryo (parthenapogamous) within the venter of archegonium. *n*, neck of archegonium. *a*, antherozoid; the arrow → shows the direction of the apex of the prothallium. In the epibasal half two nuclei are shown separated by the (invisible) median wall. In the hypobasal half two nuclei are shown separated by a wall parallel with the basal wall *BB*.

Fig. 30. Transverse section of the epibasal half of a young embryo showing the median and transversal walls. The section was taken transversely through the prothallium at right angles to its axis of growth.

Athyrium Filix-fœmina var. *unco-glomeratum*, Stansfield.

Fig. 31. Nucleus from prothallial cell. *nu*, nucleoli. × 1500.

Athyrium Filix-fœmina.

Fig. 32. Spore-mother-cells with the heterotype chromosomes. × 2250.

Fig. 33. Prothallial cell with nucleus. × 1500.

Fig. 34, *a*, *b*, *c*. Spermatocytes with stages in the differentiation of antherozoid. × 1500.

Lastrea Filix-mas.

Fig. 35. Oosphere in archegonium showing fertilization stage. *a*. the antherozoid in the oosphere; *a* 1, antherozoids that have failed to penetrate the oosphere; *B*, the 'body' in the oosphere. The track of the antherozoid in the oosphere is seen to pass across it. *N*, neck of archegonium.

Scolopendrium vulgare var. *crispum Drummondae.*

Fig. 36. Oosphere within the venter of the archegonium. The nucleus shows tendency to amitosis. *v*, the nucleus of ventral canal-cell.

Fig. 37. Young embryo in section. *ar*, part of the archegonium neck.

Lastrea pseudo-mas var. *polydactyla*, Wills.

Fig. 38. Nuclear division of young archesporial tissue. × 1500.

Figs. 39, 40. Heterotype mitosis in spore-mother-cell. × 1500.

Fig. 41. Nucleus in mitosis from prothallium. × 1500.

Fig. 42. Nucleus in equatorial plate stage. In this and the preceding figure the postmeiotic number of chromosomes are shown. × 1500.

Fig. 43. Nucleus, in same stage as the last, from prothallial cell, but containing increased number of chromosomes due to fusion with a nucleus of adjacent cell. × 1500.

Fig. 44. Nucleus in same stage from cell of embryo. × 1500.

PLATE XIX.

Figs. 45, 46, 47, 48, 49, 50, 51 illustrate stages in the migration and fusion of nuclei from adjacent prothallial cells. The empty cell is drawn, but the surrounding cells, every one of which contains a nucleus, are not included in the figures: the figures are all drawn to a magnification of 1500 diam., and are reduced to ½ size.

Figs. 52, 53. Unusual types of nuclear migration. In Fig. 52 three nuclei have collected into one cell; in Fig. 53 a nucleus is seen passing from one cell, traversing the next, and entering the third cell beyond.

Figs. 54, 55. Apogamous outgrowth with tracheids arising from the prothallium.

Fig. 56. Embryo formed from the apogamous peg. The prothallium shows tracheids in the anterior end.

Lastrea pseudo-mas var. *polydactyla*, Dadds.

Fig. 57. Dividing nucleus from young archesporial tissue. × 1500.

Fig. 58. Heterotype mitosis in spore-mother-cell. × 1500.

Fig. 59. Mitosis in the antheridial rudiment. The antheridium arises as a small pouch-like upgrowth from the prothallial cell, shown in the Fig. *pr*. × 1500.

Fig. 60. Prophase of mitosis in embryo. × 1500.

Fig. 61. Migration of nuclei in prothallium. Note the wall separating the cell is breaking down.

Fig. 62. Archegonium. *v.c.c.*, ventral canal-cell; *b*, the 'body' in the oosphere.

PLATE XX.

Fig. 63. The central cell (oosphere) with 'body' (*b*), and the ventral canal-cell, (*v.c.c.*) (more highly magnified than the last figure).

Fig. 64. The apogamous protuberance (*sp*), situated close to an abortive archegonium (*Ar*) (the latter is cut obliquely).

Fig. 65, *a* and *b*. Two consecutive sections cut through older sporophytic growth (*sp*). In Fig. 65 *a* the apex of the root, in Fig. 65 *b* the adjacent archegonium, is shown.

Nephrodium pseudo-mas var. *cristata apospora*, Druery.

Fig. 66. Edge of pinna with aposporous prothallial outgrowth.

Fig. 67. End of pinna with brush-like outgrowth of marginal cells.

Fig. 68. End of pinna with marginal (*m*) and with superficial (*s*) prothallial outgrowths.

Fig. 69. Prothallial cell and nucleus. × 1500.

Fig. 70. Mitosis in nucleus of prothallial cell. × 1500.

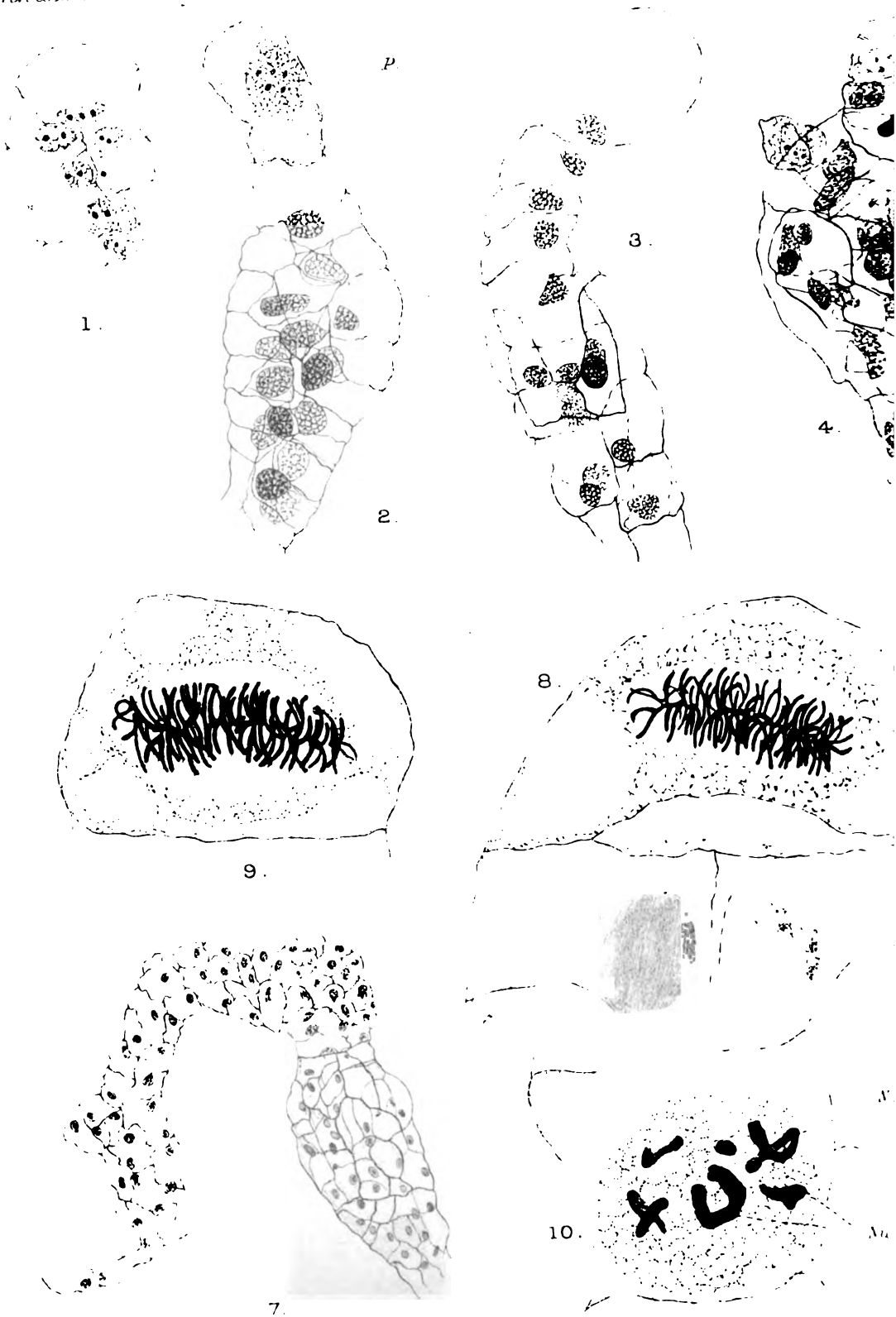
Fig. 71. Somewhat later (equatorial plate) stage in mitosis of prothallial nucleus. × 1500.

Figs. 72, 73. Nuclear division in Embryo. × 1500.

Fig. 74. Section through a young apogamous outgrowth on the prothallium.

Fig. 75. Section through an older apogamous embryo cut obliquely, and showing the apex of the root.

Fig. 76. Aposporous prothallium and apogamous embryo still attached to it.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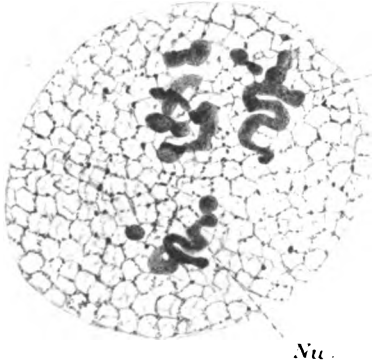

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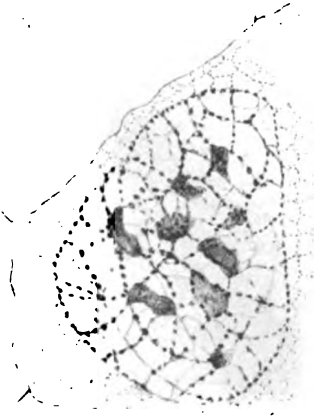

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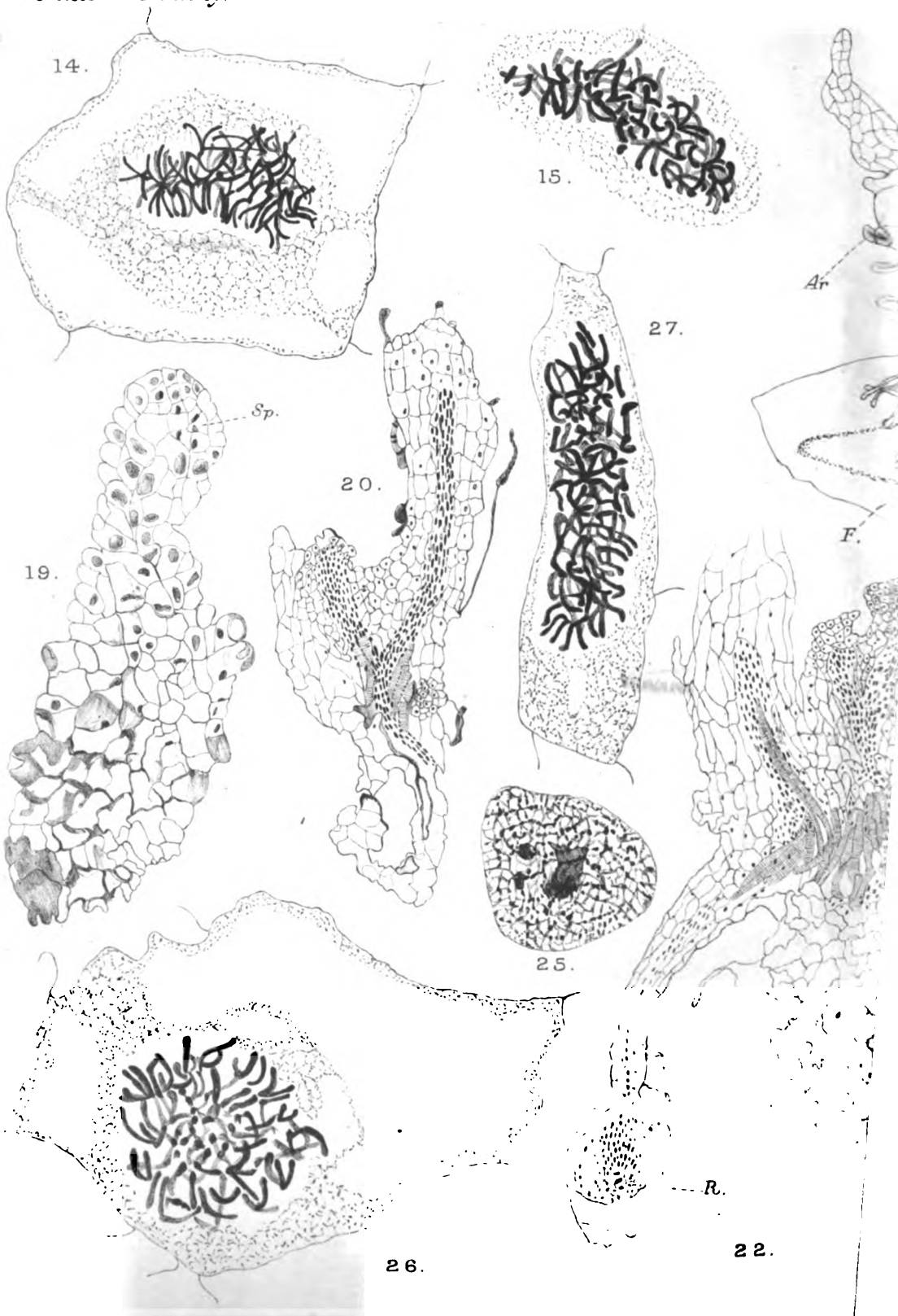
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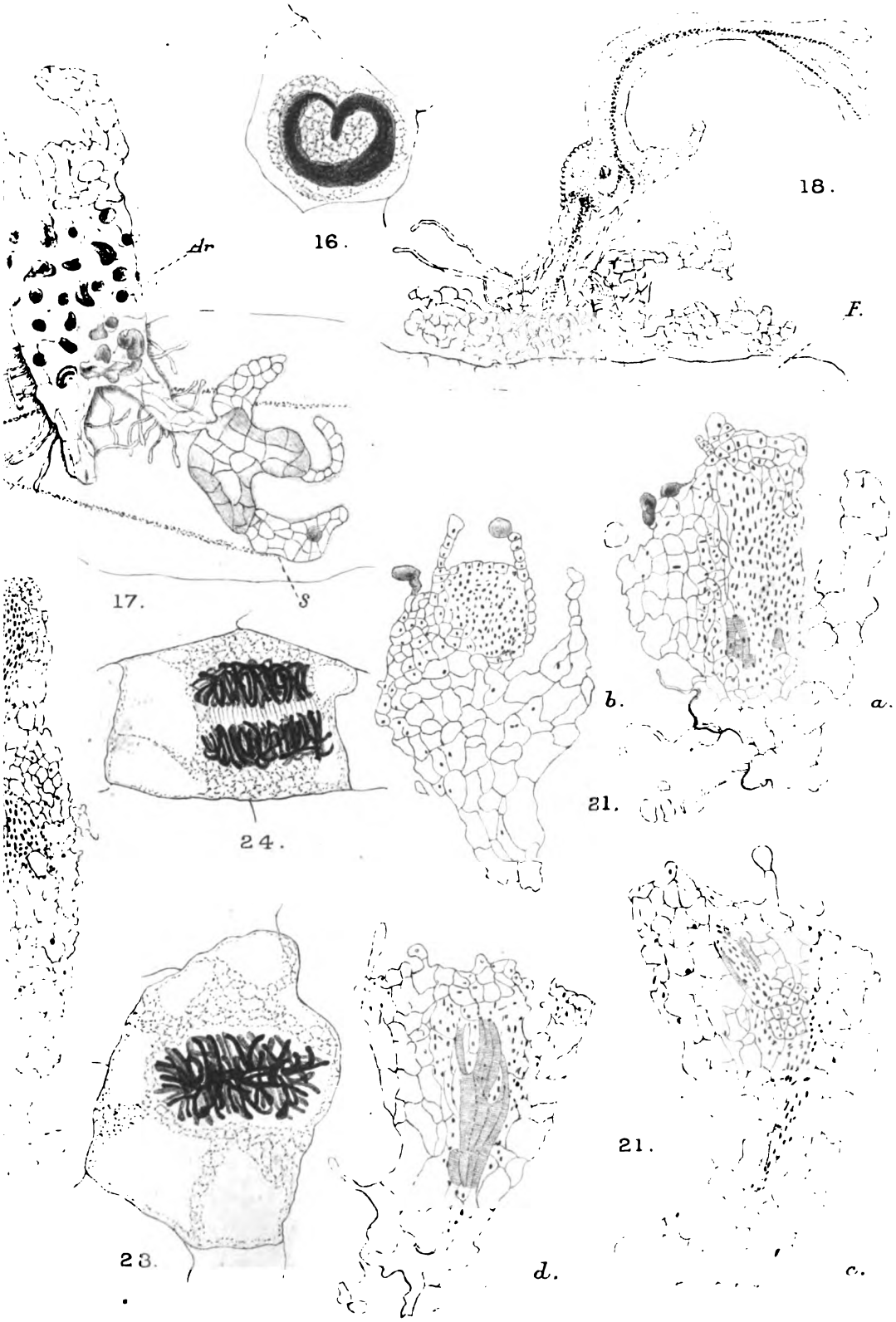
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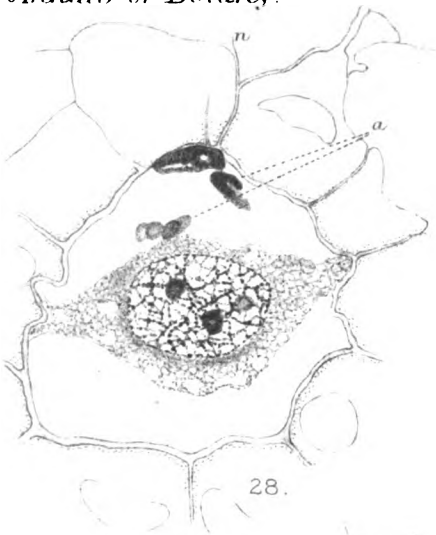

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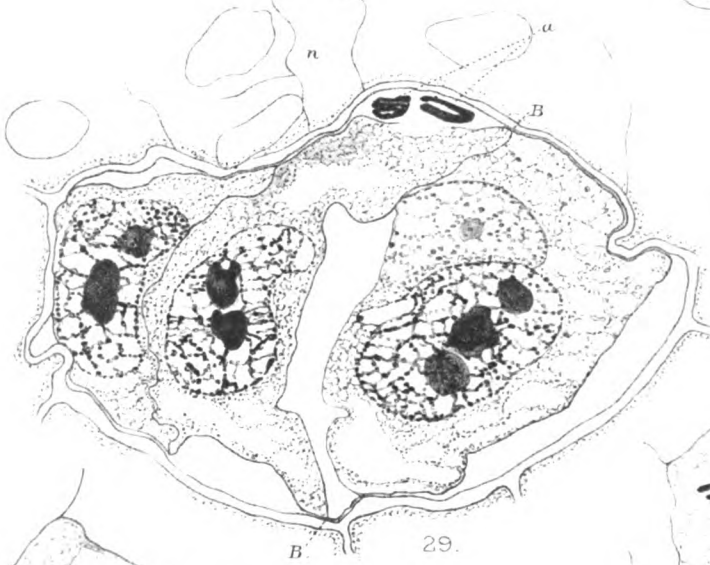

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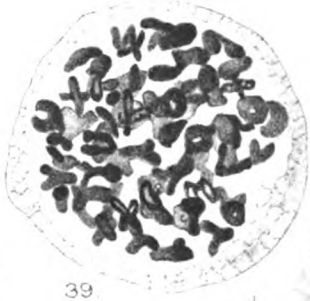

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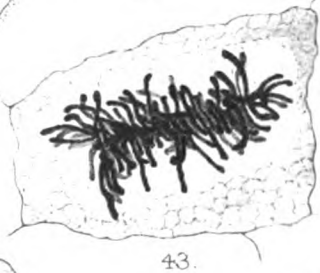

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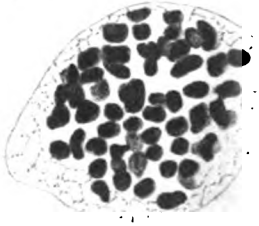

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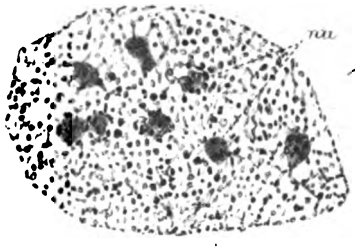

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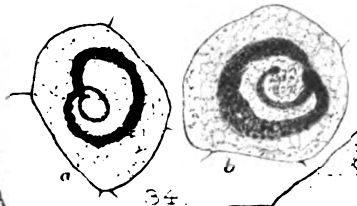

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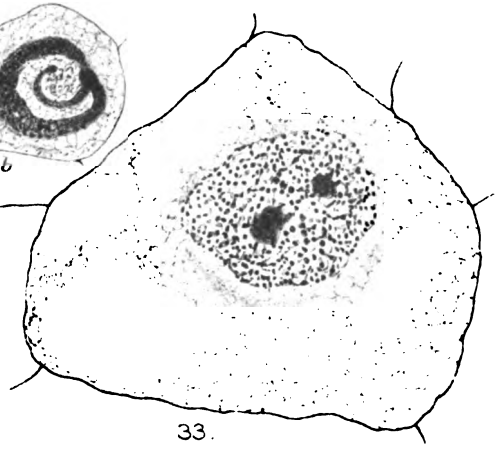

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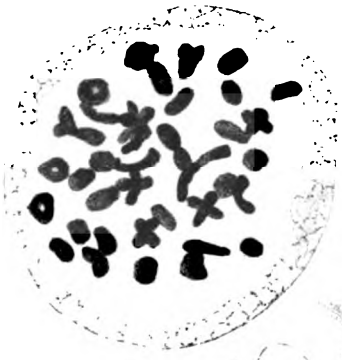

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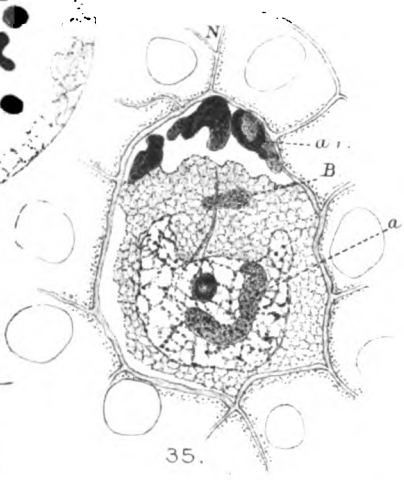

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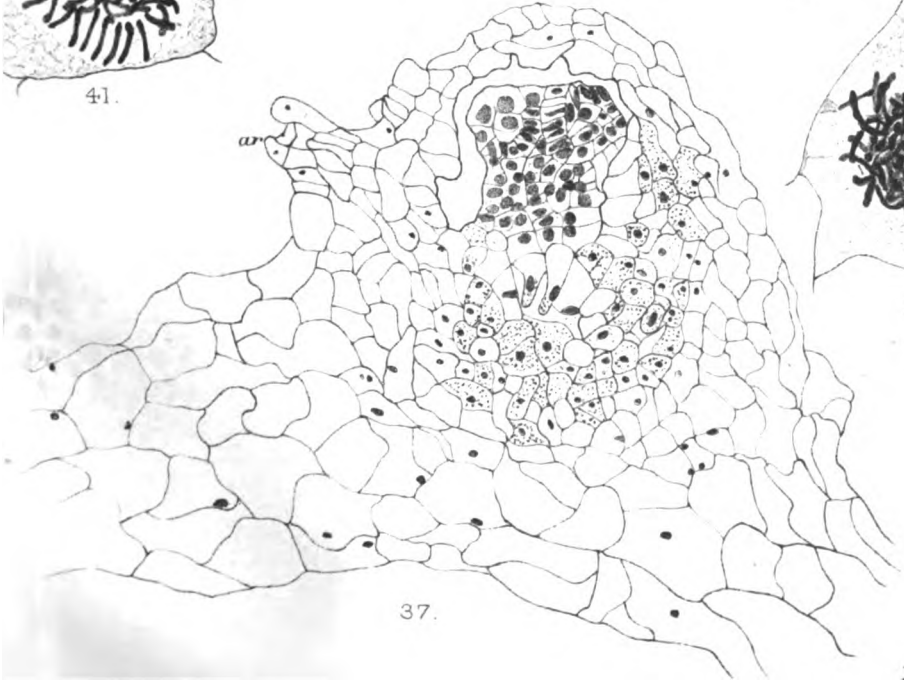

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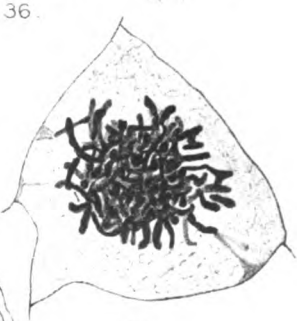

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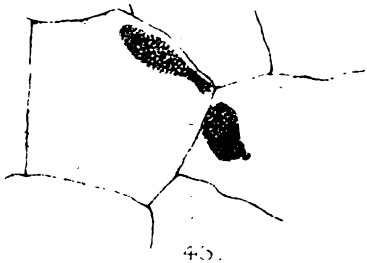

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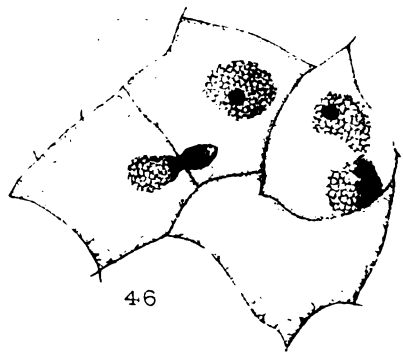

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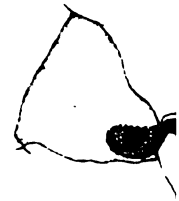

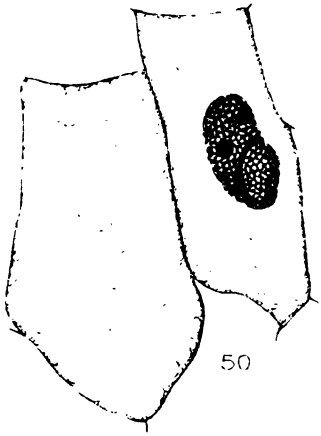

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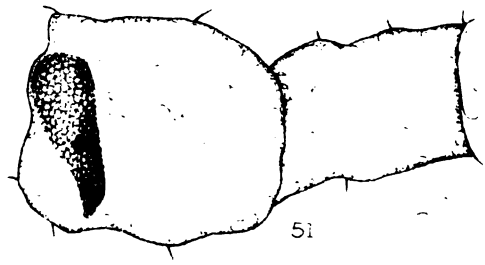

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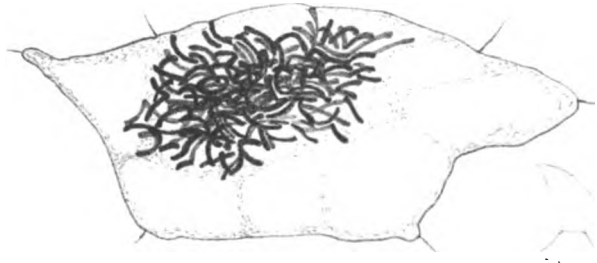

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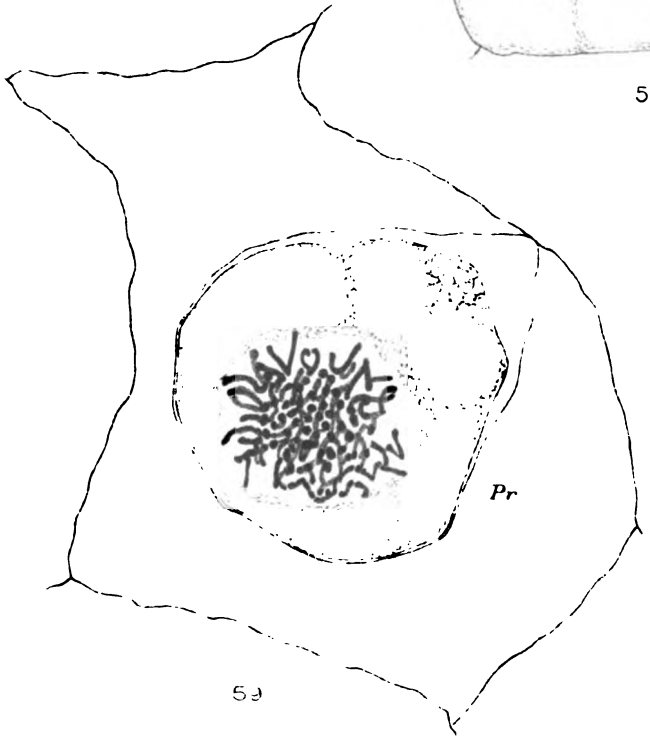

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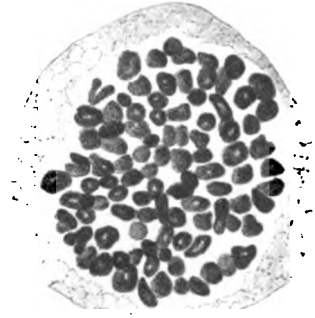

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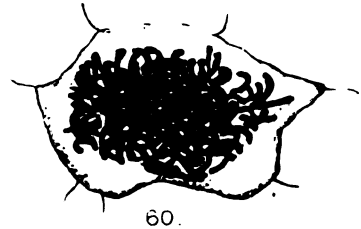

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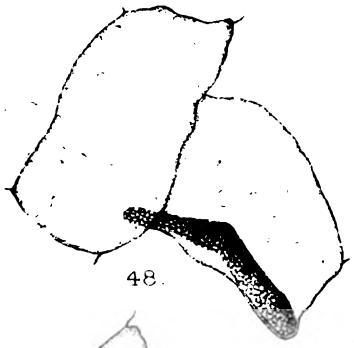
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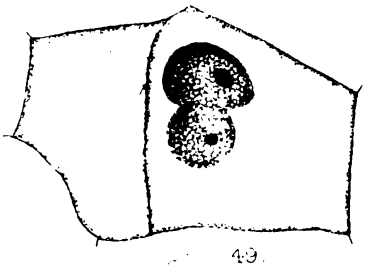
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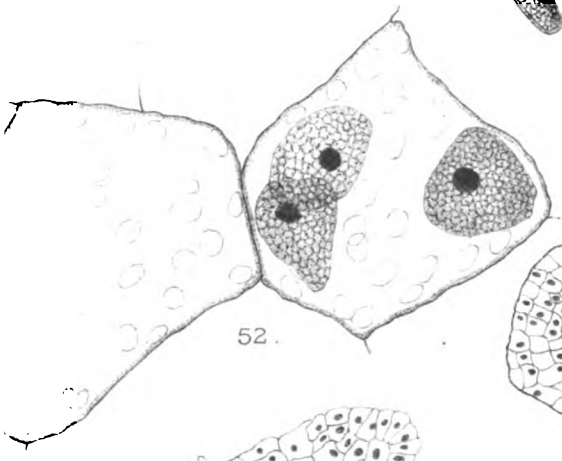
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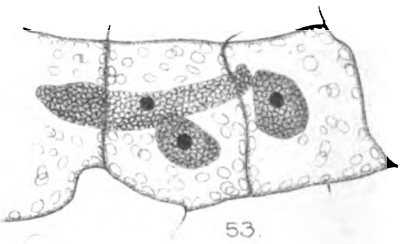
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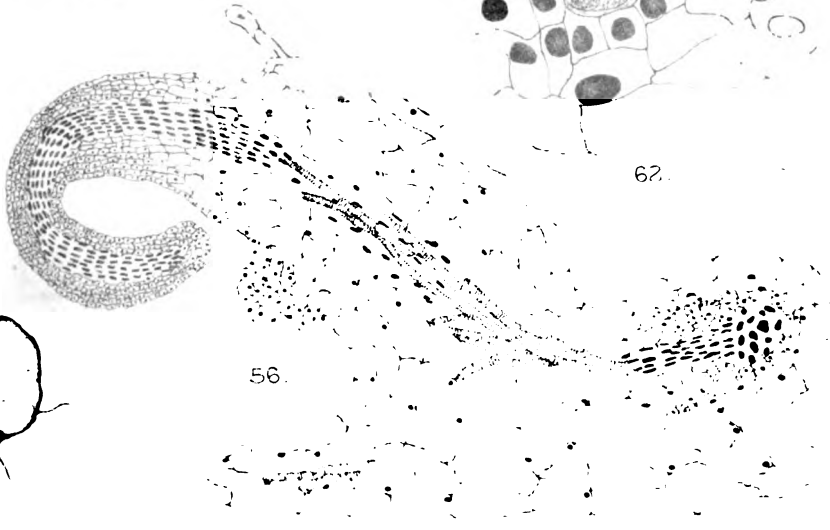
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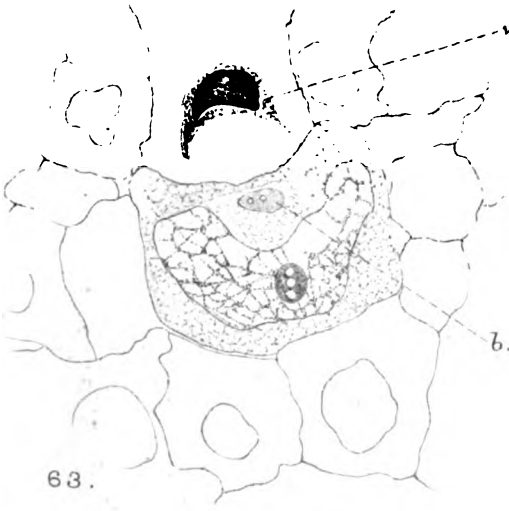

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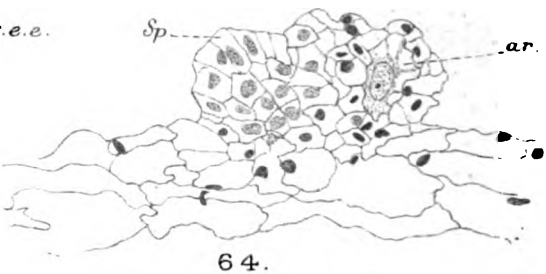

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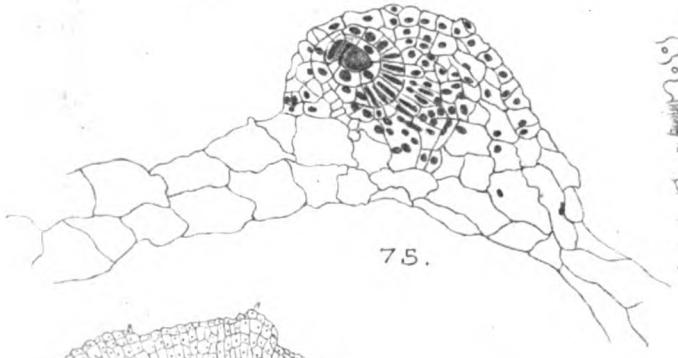

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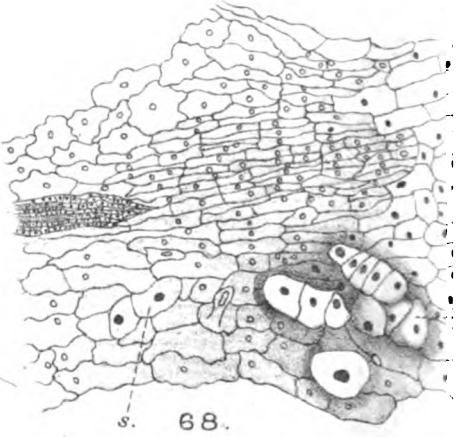

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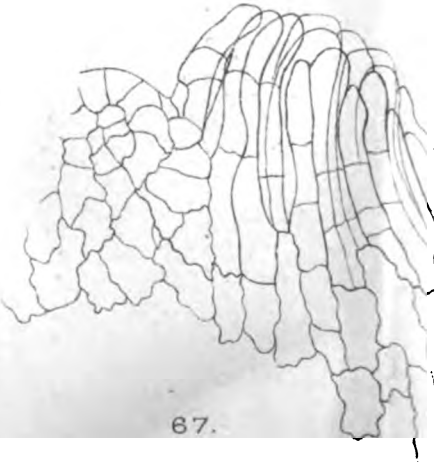

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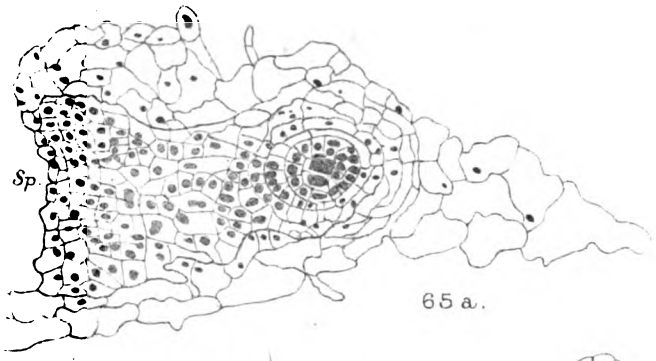

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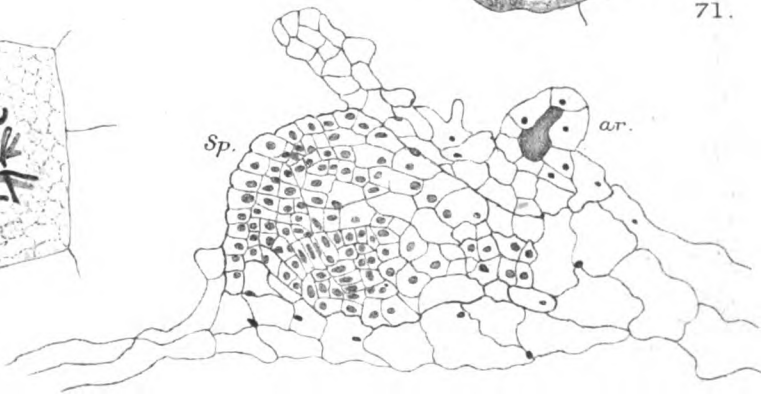

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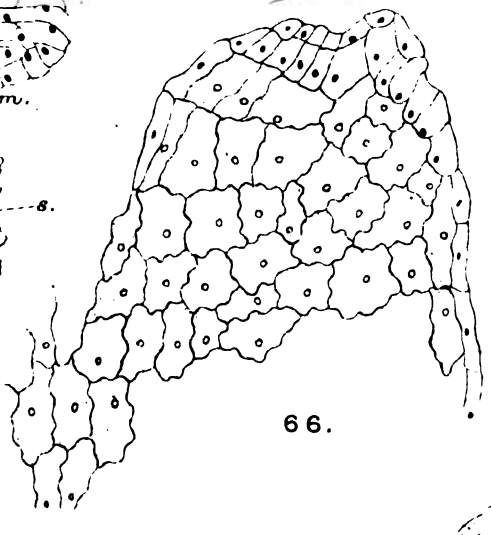

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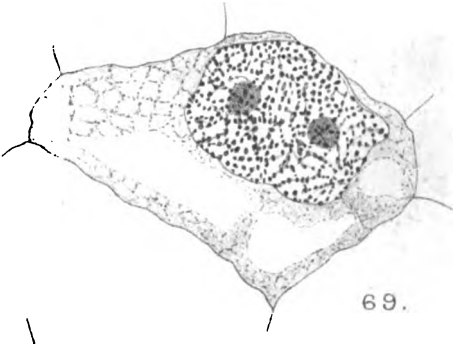

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On the Sporogonium of Notothylas.

BY

WILLIAM H. LANG, M.B., D.Sc.

Botanical Department, University of Glasgow.

With Plate XXI.

THE only indication of direct relationship between the Anthocerotaceae and any other group of Liverworts has been afforded by Leitgeb's[1] study of the sporogonium of *Notothylas*. This extended and careful investigation was carried out with special thoroughness so far as the material of a number of species allowed, and Leitgeb's observations must be given full consideration, even if regarded as insufficient to support the view of a genetic connexion between the Anthocerotaceae and the Jungermanniaceae, which he only put forward tentatively. Recent researches on satisfactory material of *Notothylas orbicularis* have shown that the well developed columella of this species is not (as Leitgeb had suggested was the case throughout the genus) due to secondary differentiation within the sporogenous tissue, but is defined by the first periclinal divisions in the embryo, as in other Anthocerotaceae. The lack of agreement between the facts of development in this species and the conclusions of Leitgeb leaves us in a state of uncertainty on a number of points which can only be cleared up by the reinvestigation of other species. The present paper is a contribution towards this.

A brief outline of the progress of investigation and opinion on the subject will serve to make the points at issue clear. *Notothylas* as founded by Sullivant[2] was described as having a columella in its small capsule. Shortly afterwards another form was found by Milde[3] in Germany, and, mainly on the absence of a columella, was placed by him in a new genus as *Chamaeceros fertilis*. Gottsche[4] in 1858 critically discussed the question and collected and grouped the known species. He found that many capsules of Milde's plant possessed a columella, and did not regard it as even specifi-

[1] Untersuchung über die Lebermoose, Heft v, 1879.
[2] Musci and Hepaticae of the United States, East of the Mississippi River. New York, 1856.
[3] Nova Acta, xxvi, 1856, p. 167. [4] Bot. Zeit., 1858, Beilage.

[Annals of Botany, Vol. XXI. No. LXXXII. April, 1907.]

cally distinct from *N. valvata*, Sull. He explained the absence of a columella as due to the latter sometimes separating into its constituent cells. In the following year Milde [1] reinvestigated the genus, and (though his drawings do not appear to bear this out) stated that the breaking down of the columella took place sooner or later in every case. The work of these earlier investigators, which was carried out from the systematic standpoint by the dissection of dried material, resulted in the recognition of the fact that a columella might or might not be demonstrable in the mature capsule of any of the species.

The work of Leitgeb [2], since it took the development of the sporogonium into account, was on another plane, but was hampered by the lack of young stages. He had fresh material of *N. fertilis*, and herbarium material of *N. valvata*, *melanospora* and *Breutelii*, and confirmed the fact that in nearly mature sporogonia of the same species the columella might be present or wanting. By extending his observations to the basal region of the capsule, where the tissue was still continuous, he showed that the columella was really absent, and did not merely dissociate into elater-like cells. When present it was often thin and composed of cells similar to the elaters, but in other cases was as well differentiated as in capsules of *Anthoceros*. From the study of a few young sporogonia of *N. fertilis* and *N. Breutelii* he came to the conclusion that, while the first divisions of the embryo were like those of *Anthoceros*, the central group of cells defined by the first periclinal walls gave rise in *Notothylas* to the sporogenous tissue, within which a sterile columella was subsequently differentiated. While he recognized the possibility that when the columella was well developed and distinct it might have originated as in *Anthoceros*, he regarded it as unlikely that such differences in the laying down of the sporogenous tissue should exist within the genus.

The work of Campbell [3] and Mottier [4] was done on *N. orbicularis*. The capsules were found in all cases to have a well-developed columella, and the delimitation of this in the embryo and the origin of the archesporial layer from the amphithecium proceeded as in the other Anthocerotaceae. Campbell regarded it as unlikely that the other species should differ so radically from the one he investigated as would be the case were Leitgeb's surmise correct.

If the inferences as to the uniform development of the sporogonium throughout the genus, made on the one hand by Leitgeb, and on the other by Campbell, are put on one side, the observations of the various investigators are not readily combined into a consistent account of the sporogonium of *Notothylas*. It is clear that a columella developed as in *Anthoceros* may be present, and this is doubtless the origin of the well-defined columella

[1] Bot. Zeit., 1859, p. 33.
[2] loc. cit.
[3] Mosses and Ferns, 1st Ed., p. 140.
[4] Annals of Botany, xxxii, 1894, p. 391.

which Leitgeb observed in some examples. But this does not appear to justify putting on one side the recorded cases in which the columella was thinner and less distinct, or the records of its complete absence in many sporogonia. Leitgeb found that in sections across the basal region of such capsules the centre was occupied by a uniform mass of sporogenous cells. This observation is independent of his explanation of the origin of the sporogenous tissue from the endothecium alone; as based on few and possibly abnormal embryos this is open to doubt, though even here the facts he records are not to be lightly put aside.

The study of the form of *Notothylas* to be described below appears not only to provide some additional facts, but to show how the conflicting statements, which from the foundation of the genus have been made as to the presence or absence of a columella, may be reconciled. My material was collected in the Singapore Botanic Gardens, and since it was in abundant fruit, the small supply preserved in spirit has sufficed for a fairly complete study of the development and structure of the sporogonium.

The form in question has dark spores, which are muriculate on the convex face, and thus belongs to the section *Acantho-Notothylas*, and differs from the species of *Eu-Notothylas* (including *N. javanicus* from the same geographical region), in which the spores are smooth. Specific differences in the genus are slight and indefinite, and from published descriptions and figures this form at first seemed distinguishable from *N. Breutelii*. Comparison with specimens from Cuba[1] showed, however, such close agreement in all definite features that there seems no ground for distinguishing the plant from Singapore even as a variety from *N. Breutelii*. This species, which was first described as *Anthoceros Breutelii* in the Synopsis Hepaticarum, has been examined with regard to the presence or absence of a columella by Gottsche, Milde, and Leitgeb. The material examined by the two former observers showed as a rule a well-marked columella, between which and the wall the elaters extended. Milde, however, believed the columella to ultimately disintegrate, since he sometimes found it intact, sometimes only larger or smaller fragments, while in other cases no trace of it was to be found but a short stump-like portion at the base of the capsule. He figures a very complete columella. Leitgeb found a more or less perfect columella in some sporogonia. In sections of the base of other capsules no sterile columella could be distinguished, and on the evidence afforded by several embryos he regarded the whole sporogenous mass as derived from the endothecium and the columella as differentiated within it. Thus this particular species illustrates the difficulty which has been experienced in the genus as a whole of attaining a clear statement of the facts of structure of the sporogonium.

[1] I am indebted to Professor Weiss for the opportunity of examining authentic specimens in the Herbarium of the Victoria University, Manchester.

The characters of the gametophyte need only be briefly referred to for the purpose of systematic comparison and as introductory to the more detailed account of the sporogonium. The plants grew on the surface of a path, the individual thalli closely overlapping one another. The form of a single complete plant developed from the spore was not ascertained, since the branches were mostly in continuity with older underlying portions. The individual lobes or branch systems attained a length of 5–10 mm., and widened out from a narrow base to a breadth of 12 mm. or less. The thallus thus assumed the usual suborbicular form, and the numerous growing-points in the anterior margin were separated by rounded or spathulate middle lobes (Fig. 1). For the most part the thallus, which had no definite midrib, was six to eight cells thick. Each of the smaller cells of the limiting layer above and below contained a single large chloroplast. The internal cells were large and clear, and some of them contained mucilage; intercellular spaces containing mucilage were absent. In the middle lobes the internal tissue became reduced to a single layer of mucilage cells, and at the extreme margin was wanting, the lobe being composed of two layers of cells. The apical region resembled that of *N. orbicularis*, and numerous mucilage pits, some of which were later occupied by large oval colonies of *Nostoc*, were present. The antheridia and archegonia were situated behind the apical regions on the same individuals. Usually a pair of large antheridia occupied the antheridial cavity, but sometimes three were found, and occasionally the antheridium was solitary. The inconspicuous sunken archegonia resembled those of *N. orbicularis*. Fertilization appears to take place when the archegonia are close to the apex, and as a rule further growth of the thallus is slight, so that the sporogonia are situated close to the margin of the thallus (Figs. 1, 2). Occasionally sporogonia were found further back on the thallus.

The calyptra enclosing the sporogonium was directed forwards, parallel to the surface of the thallus (Figs. 1, 4, 5). It measured from 1·5 to 2·5 mm. in length by about ·75 mm. across. The side turned away from the thallus bore a number of longitudinal wing-like ridges, and the calyptra was thicker on this side than the other. The mature sporogonium usually projected only a short distance from the calyptra. Its length varied from 2 mm. to 4·25 mm., but dwarf forms, like those mentioned by Leitgeb, were also seen, in which the total length was under 1 mm. (Fig. 3). The bulbous foot, the superficial cells of which grew out into processes, was separated from the cylindrical slightly curved capsule by a slender stalk (Fig. 4). The mature capsule itself was from 1·5 to 3·75 mm. long. The superficial cells of the wall were more or less rectangular, and their anticlinal walls were strongly thickened and of a brown colour. The line of dehiscence was clearly marked, the margins of the two valves being formed of narrower, reddish-brown cells. The opening of sporogonia attached to the plant was

not observed, but most of the capsules were not quite mature. The occurrence in the underlying soil of intact capsules isolated by decay suggests, however, that in many cases they may not open. It is indeed difficult to see how the small part of the capsule projecting from the calyptra can suffice for effective dehiscence. The cavity of the capsule was occupied for the greater part of its extent by a uniform mass of spores and elaters, a sterile columella being completely absent or only present at the base. The spores measured 40–45 μ in diameter: the wall was dark and the convex outer face was studded with short, blunt, almost black projections. The individual sterile cells or elaters, which at first were united into flat transverse plates, measured about 40 μ x 30 μ, and had thin walls with yellowish-brown thickenings in the form of more or less complete rings.

The general appearance of an almost mature sporogonium in median longitudinal section is shown in Fig. 4, and Fig. 5 represents a similar section of a smaller and slightly less advanced sporogonium under a somewhat higher power. These figures, together with Figs. 6 and 7, which represent median longitudinal and transverse sections from mature capsules under a high power, demonstrate the absence of a sterile columella from the greater part of the capsule. The cavity of the latter is seen to be filled with a reticulum of sterile cells, in the meshes of which the spores lie. The structure is in fact that described and figured by Leitgeb as occurring in some cases in *Notothylas*, although, as will be shown below, its origin in development is different from what he supposed. When a columella was present it only extended a short distance from the base, and its diameter varied in different capsules.

From this general survey of the structure of the mature sporogonium we may return to trace its development. A sufficient number of young embryos were found to indicate clearly the course of the early segmentations of the fertilized ovum. In Fig. 8 a young embryo is shown in median section as it lies in the venter of the archegonium, and the commencing growth of the surrounding tissue to form the calyptra is evident. The first division is in the line of the axis of the archegonium (Fig. 9 *a*). In the next stage found the embryo consisted of three tiers of four cells each (Fig. 9 *b*); this stage has been found in the embryogeny of all Anthocerotaceae that have been investigated. Comparison with succeeding stages shows that here, as in *Anthoceros*, the two lower tiers go to form the foot, while the capsule and stalk originate from the uppermost tier. The periclinal divisions in the four cells of the latter separate the endothecium from the amphithecium, as in *Anthoceros* (Figs. 8, 9 *c*). After some anticlinal divisions have occurred, and the young capsule has increased in size, the archesporium is cut off from the amphithecium. This begins at the summit of the embryo and extends to the cells below, but, as Fig. 10 shows, not to the base of the region derived from the uppermost tier of cells. The

limits of foot, stalk, and capsule are thus clearly established at this stage, and continue to be traceable throughout the further development of the sporogonium. In the slightly older embryo seen in median section in Fig. 11 the capsule has grown considerably. The whole course of the segmentation of the embryo thus agrees with the very uniform type for the Anthocerotaceae. In these young capsules the regions corresponding to the wall, the archesporium (derived from the amphithecium), and the columella in other Anthocerotaceae, can be recognized. All these regions are present in the zone just above the stalk, from which the intercalary growth proceeds.

The difference between the mature capsule of this form of *Notothylas* and the typical capsule of the Anthocerotaceae depends on the different fate of the cells of the endothecium in the two cases. In *Anthoceros* this is devoted to the formation of the sterile columella. In this *Notothylas* it can only be called the columella on comparative grounds, for, as intercalary growth proceeds, the cells of the endothecium, as well as those of the amphithecial archesporium, give rise to sporogenous tissue. In this way the capsule, as has been shown above, comes to be filled with a uniform mass of spores and elaters.

The differentiation of the tissues within the wall can be followed even in sections of advanced sporogonia, so that the absence of some of the intermediate stages from the material has been of little importance. Fig. 12 is a detailed drawing of the median section of the sporogonium represented in Fig. 5, and includes the region from the stalk to the base of the capsule, where the distinction of elaters and spore-mother-cells is becoming established. In the stalk the endothecium appears as two rows of cells bounded by the amphithecium, consisting of a single layer. Just above this the amphithecium is seen to consist of two layers, the outer of which is continuous when traced upwards with the wall of the capsule; the inner can be followed upwards as a definite layer adjoining the endothecium, and is the archesporial layer. Though different in origin, the four rows of cells seen within the wall in Fig. 12 behave alike. Their cells undergo an ultimate transverse division, the upper segment forming one or more spore-mother-cells, while the lower gives rise to sterile elaters. In the figure the spore-mother-cells are shaded. Comparison with Fig. 6 will make it clear how the reticulum of sterile cells found in the mature capsule is derived. The relation of the uniform sporogenous tissue to the meristematic zone below in Fig. 12 demonstrates its origin from series of cells which correspond in other Anthocerotaceae, both to the archesporium and the columella. Further evidence of the complete absence of a sterile columella from such capsules is afforded by transverse sections of the basal region. In Fig. 13 the limits of the four rows composing the endothecium and the surrounding groups of cells derived from the archesporium can be traced in the uniform

sporogenous tissue. These limits continue to be traceable, even in the zone at which the spore-mother-cells are differentiated, and Fig. 14 *a* and *b*, which are two successive sections of the sporogenous tissue at this level, afford additional proof of the sporogenous nature of the endothecium.

For the greater part of the intercalary growth in all the sporogonia examined the endothecium and archesporium behave alike as described above. In many sporogonia a sterile columella appears towards the close of growth. This results from the intercalary meristem of the endothecium becoming in whole or part sterile. The change is apparent as soon as the sporogenous cells acquire the dense appearance by which they contrast with the cells of the wall of the capsule. When one or more quadrants of the endothecium have become sterile, they also stand out in contrast to the sporogenous cells (Fig. 15 *d*, *e*; Fig. 16). The series of transverse sections in Fig. 15 illustrates a case in which one quadrant of the endothecium had become sterile, while the other three were sporogenous. This series extends from the stalk to the region where the elaters and mother-cells are distinguishable, and may be compared with the longitudinal section in Fig. 12. In the longitudinal section through the base of another capsule (Fig. 16) a short columella, derived from the whole thickness of the endothecium, is shown. Such short columellas were found at the base of some fully mature capsules, and did not appear to undergo any disintegration into separate cells. In the numerous capsules examined the sterilization only supervened shortly before the completion of intercalary growth. The arrest in growth was in no way dependent on decay of the stalk, and Leitgeb's suggestion to this effect seems to be without proper foundation. It seemed, on the other hand, to be associated with the maturing of the spores at the tip of the capsule. When these have acquired their dark-coloured walls, a change which extends downwards towards the base of the capsule, active growth appears to cease.

We may sum up the chief features of the development and structure of the sporogonium in this form, by saying *that the embryogeny conforms to the usual type for the Anthocerotaceae, but that the endothecium, instead of being devoted to the formation of a sterile columella, forms sporogenous tissue for the greater part of the intercalary growth of the sporogonium. In a considerable proportion of cases, however, it produces sterile tissue towards the close of development.*

Other investigators have shown that in *N. Breutelii* there is sometimes a complete sterile columella, and this doubtless arises by the endothecium being sterile from the first. The range of variation in the development of the internal tissues of the embryo may fairly be assumed to be dependent on physiological factors which vary from individual to individual, and not in this case to be of value as an indication of specific difference. That the majority of the sporogonia in a single small collecting should show the same

degree of development of the columella is quite consistent with this. Not only do Leitgeb's observations on this species agree with what has been shown above as to the complete absence of a sterile columella in some capsules, but his few observations on arrested embryos suggest that a further grade of simplification may occur, in which only the endothecium is sporogenous. Were no amphithecial archesporium cut off, the sporogenous tissue would be traceable downwards to four rows of cells in the stalk, as he describes and figures it. It would be of great interest to have these observations, the possibility of which must be admitted, verified. Unfortunately satisfactory preparations were not obtained of the two dwarf capsules found in my material, and in all the other embryos and sporogonia examined it was clear that both endothecium and amphithecium had contributed to the sporogenous tissue. If such extreme forms occur they would seem to be infrequent and exceptional.

The position of *Notothylas* among the Anthocerotaceae, and of the group as a whole in relation to the other Hepaticae, may in conclusion be briefly considered in the light of the facts as at present known. The point that has emerged from the study of this form of *N. Breutelii*, that the endothecium must be regarded as potentially sporogenous, is of considerable theoretical interest. It increases the justification for considering the central group of cells, which in all other Anthocerotaceae is wholly devoted to the formation of a sterile columella, as the original sporogenous tissue and the amphithecial archesporium as of secondary origin. The duty of producing spores would seem to have been transferred from the central to a more superficial set of cells. This may, I think, be regarded on comparative grounds as probable, whether or not sporogonia are met with in which the amphithecial archesporium is absent. Leitgeb's deduction that the columella was to be looked on as originating by gradual differentiation within the sporogenous tissue does not, on the other hand, properly represent the facts. The causes of the change from the fertile to the sterile condition of the products of the endothecium must be looked for in influences acting on the primary meristematic tissue of the embryo, or on the intercalary zone of secondary meristem. The idea of a grouping of elaters in a central position to form the columella is not in this case in accord with the facts. The diverse results of the development of the similar embryos of the Anthocerotaceae suggest the influence of nutritive factors acting on the young embryo when it is enclosed and dependent like a foetus in the uterus on the tissue of the gametophyte.

The interest of the potentially sporogenous nature of the region usually developed as columella is independent of the question as to whether the species of *Notothylas* are to be regarded as primitive or reduced in comparison with the Anthocerotaceae with larger sporogonia. I am inclined to regard the evidence as pointing to their being reduced forms. The appear-

ance of the archesporial layer derived from the amphithecium, even when the central cells are fertile, suggests an origin from forms with a sterile columella. In other words, the fertility of the endothecium in some cases seems better explained as the resumption of a lost power than as a persisting primitive feature. An origin from forms with a larger capsule seems also to be indicated by the preparations for splitting of the wall of the capsule into two valves extending to the base, even though the sporogonium is usually almost enclosed by the calyptra and effective dehiscence rendered impossible. I incline to look upon *Notothylas* as a rather artificial genus, including a number of forms derived by reduction in size of sporogonia from one or more *Anthoceros*-like forms. Accompanying the reduction are the changes in development of the regions of the embryo; these, though becoming manifest in reduced forms, may have an important bearing on the morphology of the Anthocerotaceous type of sporogonium.

The possibility or probability that the ancestral forms of the Anthocerotales had, like the other Hepaticae, a central sporogenous tissue derived from the endothecium does not lead us far in the search for the ancestry of this very natural and isolated group. In considering this question, all the peculiarities of cell structure, of the gametophyte and sexual organs, and of the sporogonium must be taken into account. There appears to be no indication at present of any natural relationship being established between the Anthocerotales and any particular series of Hepaticae. Whether they were derived from primitive forms of the same stock as the other Hepaticae, or independently, but by corresponding steps from what we provisionally assume as the unknown Algal ancestral forms, or whether on the other hand reduction has played a part in the origin of the group as we now have them, seem to be at present open questions.

EXPLANATION OF FIGURES IN PLATE XXI.

Illustrating Dr. Lang's Paper on the Sporogonium of *Notothylas*.

Fig. 1. Outline of a branch system of the thallus of *Notothylas Breutelii* bearing sporogonia of various ages. × 5.

Fig. 2. Calyptra enclosing an almost mature sporogonium, the tip of which projects: the ridges on the calyptra are indicated. × 16.

Fig. 3. A dwarf sporogonium drawn to the same scale as the one in the preceding figure.

Fig. 4. Median longitudinal section of a similar sporogonium to that in Fig. 2, showing its relation to the thallus, the regions of the sporogonium, the gradual ripening of the spores from above downwards, and the absence of a sterile columella except at the extreme base. × 20.

Fig. 5. Longitudinal section of a similar but slightly less advanced sporogonium, which is completely enclosed within the calyptra. × 45.

Fig. 6. Small portion of a median longitudinal section of a mature capsule, showing the arrangement of elaters and spores and the absence of a sterile columella. × 110.

Fig. 7. Transverse section of a similar sporogonium. × 110.

Fig. 8. Longitudinal section of a lobe of the thallus, showing an embryo in position; the endothecium and amphithecium are separated in the upper tier. × 190.

Fig. 9 *a*, *b*, *c*. Young embryos in median section, showing successive stages of segmentation. × 530.

Fig. 10. Median section of more advanced embryo, in which all the regions of the sporogonium have been laid down. × 375.

Fig. 11. Slightly older stage showing the rapid growth of the terminal group of archesporial cells. × 375.

Fig. 12. Median longitudinal section of the lower portion of the sporogonium in Fig. 5. In the upper part the wall of the capsule can be distinguished from the sporogenous tissue, the cells of which are differentiated into spore-mother-cells (shaded) and elaters. Traced downwards the central portion of the sporogenous tissue is continuous with the endothecium, while the peripheral portion comes from a layer of cells which have a common origin with the wall. × 250.

Fig. 13. Transverse section of a sporogonium at about the level *aa* in Fig. 12. The uniform sporogenous tissue (shaded) can be seen to be composed of four central groups of cells (endothecium) and a surrounding layer derived from the amphithecium. × 250.

Fig. 14. Two successive sections of the sporogenous tissue at a level corresponding to *b*, *b* in Fig. 12, to demonstrate the absence of a sterile columella and the relation between the elaters and spore-mother-cells. × 250.

Fig. 15 *a*, *b*, *c*, *d*, *e*. Successive transverse sections of a sporogonium, in which one of the four rows of cells of the endothecium is forming a sterile columella. In *d* and *e* the sporogenous cells are shaded, the sterile quadrant being left clear. The origin of the amphithecial archesporium can be followed. × 250.

Fig. 16. Longitudinal section of the basal region of an almost mature capsule, showing a short sterile columella derived from the endothecium. × 190.

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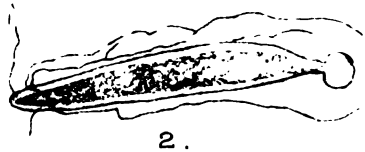

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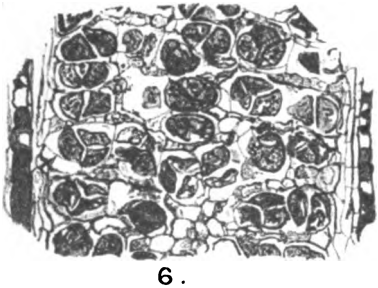

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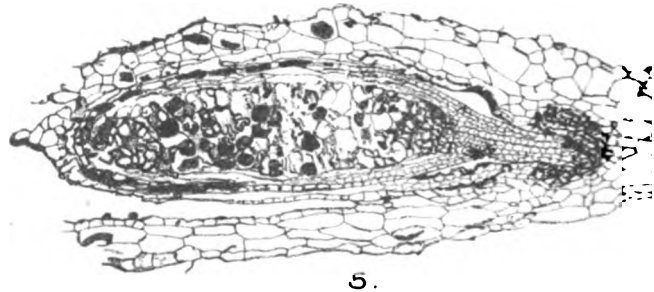

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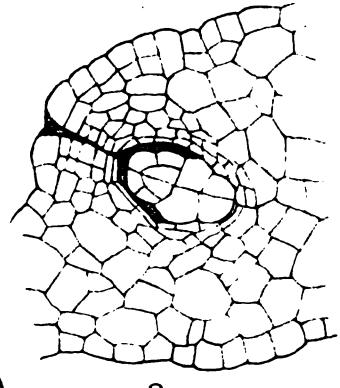

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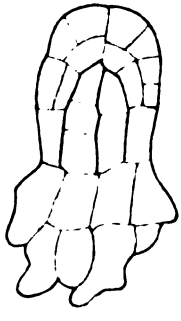

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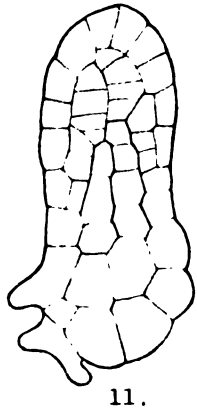

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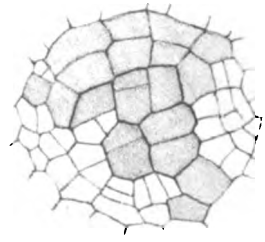

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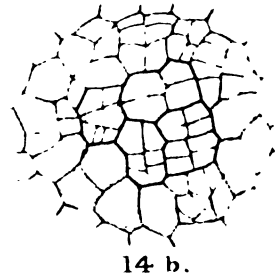

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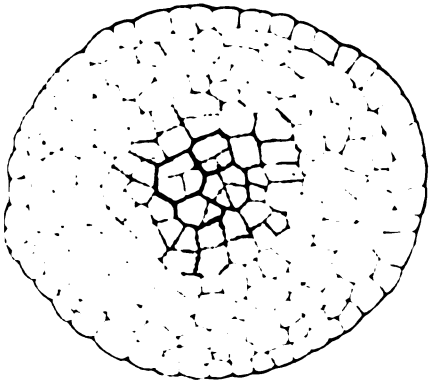

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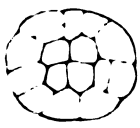

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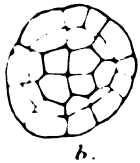

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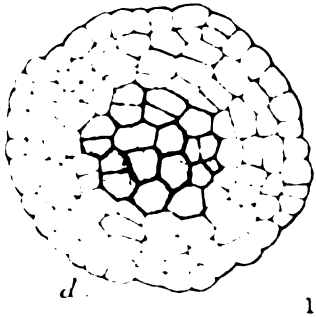

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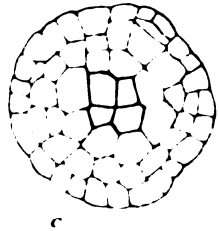

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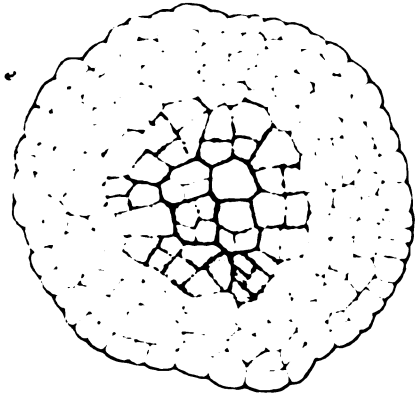

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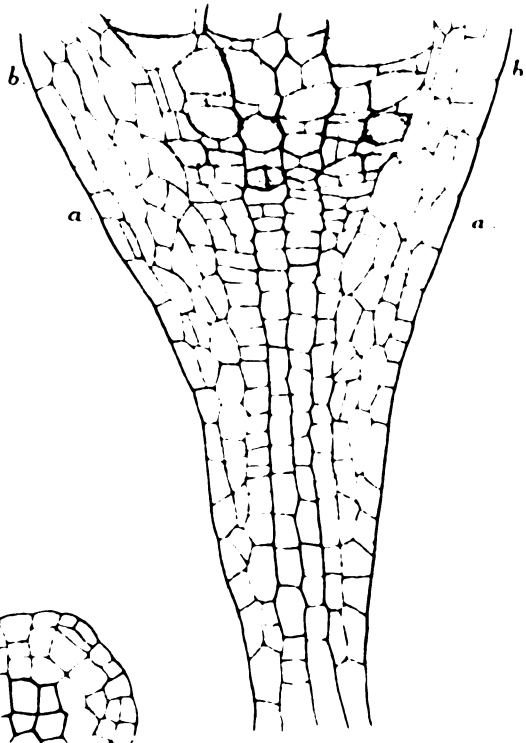

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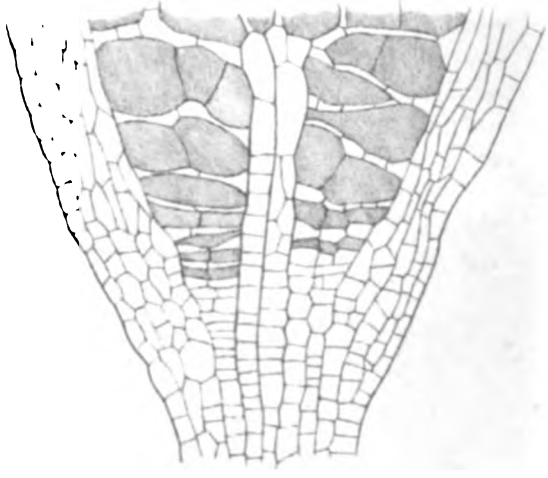

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The young Sporophytes of Lycopodium complanatum and Lycopodium clavatum.

BY

GRACE WIGGLESWORTH, M.Sc.

University of Manchester.

With Plate XXII and four Diagrams in the Text.

THE Lycopodiaceae occupy a somewhat isolated position among the plants of the present day, especially as regards their anatomy, and compared with other groups of vascular Cryptogams they have received much less attention. Their relationship to other groups is a problem still unsolved, although the careful investigation of Palaeozoic plants has considerably extended our knowledge of the Lycopodiales in general. The present paper deals only with the anatomy of the young sporophyte of *Lycopodium*. How far we can rely on the evidence afforded by the early stages in the development of the young plant, as recapitulating the history of the group to which it belongs, is a debatable question, but that ancestral characters are shown in some cases during the early stages of life seems probable, and this fact alone makes the study of young forms a highly interesting one.

A brief summary of the literature dealing with the morphology and anatomy of the stem of *Lycopodium* is given by Mr. C. E. Jones[1] in a paper read before the Linnean Society in April, 1904, in which he refers to the contributions on this subject made by Nägeli, Hegelmaier, Cramer, Sachs, Russow, Strasburger, David and Weber, Pritzel, Linsbauer, and Boodle. Mr. Jones deals chiefly with a comparison of the mature stems of different species of *Lycopodium*. He considers that the development of anatomical structure has proceeded along two lines. *L. clavatum* may be taken as a type of the group in which a series of alternating bands of xylem and phloem is developed. This form of vascular structure is characteristic of those Lycopods which have creeping stems. In the other forms, of which *L. squarrosum* may be taken as a type, the phloem is scattered through the mass of xylem in more or less isolated patches. This structure is characteristic of tropical epiphytes.

[1] Jones, The Morphology and Anatomy of the stem of the Genus *Lycopodium*. Trans. of the Linnean Soc., March, 1905.

[Annals of Botany, Vol. XXI. No. LXXXII. April, 1907.]

Mr. Jones also gives a short account of the structure of the stem of the 'seedling' of *L. clavatum*, and young plants derived from the bulbils of *L. Selago* and *L. serratum*. The structure of the roots was not worked out in any detail.

References to the anatomy of the young sporophyte of *Lycopodium* have been made previous to those of Jones. As early as 1873 Fankhauser[1] examined young sporophytes of *L. annotinum*, and found that the primary vascular strand sent off a short branch to the foot, but he apparently did not trace the changes in the stele throughout the plantlet. The most complete account of the morphology and anatomy of the young sporophyte is that given by Bruchmann[2] in his paper 'Über die Prothallien und die Keimpflanzen mehrerer europäischer Lycopodien, &c.' He describes the development of the embryo, and some important points in the structure of the young sporophytes of *L. clavatum*, giving a brief description of the root and the stem. He figures a transverse section of the stem of a young plant, showing a tetrarch arrangement of xylem and the origin of a rootlet. Other forms, such as *L. complanatum* and *Selago*, are dealt with, but not in such detail with regard to their anatomy. A brief description is given of *L. complanatum*, and a series of beautiful figures showing the external structure and attachment to the prothallus. He also describes the formation of pseudo-adventitious buds in this form, and enters fully into the branching of the roots.

The material for this investigation was obtained from Dr. Bruchmann, who had very kindly sent a series of prothallia and sporophytes, for the Manchester Museum, to Professor Weiss, and it was thought that it might serve a useful purpose to make a detailed examination of some of the duplicate material.

Two sporophytes of *L. complanatum* were examined, one still attached to the prothallus, about 3·1 cms. long; the other considerably older—about 9 cms. long (Pl. XXII, Figs. 1 and 2). Two sporophytes of *L. clavatum*, at about the same stages of development, were also examined for comparison with those of *L. complanatum*.

I. External Features.

The younger plantlet of *L. complanatum* (Fig. 1) had evidently not appeared above the surface of the ground. The short stem and first root are seen emerging from the upper side of the top-shaped prothallus, bent approximately at right angles to one another, and both considerably curved. The root has branched three or four times, but the stem is unbranched, and bears a number of small scale-like leaves, spirally arranged and at varying distances from one another.

[1] Fankhauser, Botanische Zeitung, Jan., 1873, p. 1.

[2] Bruchmann, Über die Prothallien und die Keimpflanzen mehrerer europäischer Lycopodien, &c., 1898.

The other plantlet (Fig. 2) is at a much more advanced stage of growth; there is no trace of prothallus visible to the naked eye, but the foot is plainly visible as a round warty knob at the base of the stem (*ft*, Pl. XXII, Fig. 2). The stem has branched several times, and just below the first bifurcation is an adventitious root which has already branched three times. Numerous leaves clothe the stem; the upper leaves are green and provided with vascular strands, the lower are scale-like. Just above the foot a small prominence (*p. a.* in Pl. XXII, Fig. 2) marks the position of a 'pseudo-adventitious bud,' which although of such minute size shows, in a series of transverse sections which were made in this region, dichotomy at the apex of the stem, and a root has already pushed its way through the cortex, and appears as a slight projection at the side of the bud.

II. Internal Structure.

A. *The Root System.*

(*a*) *First root.* The material at hand was not suitable for working out the embryonic development of the first root. The investigations of Treub and Bruchmann show that the primary root is absent in those forms of *Lycopodium* which they examined. Treub[1] found that in *L. cernuum* the first root arises in the interior of the embryonic tubercle; Bruchmann[2] found that in the case of *L. clavatum* it arises endogenously from the hypocotyl, and the latter states that in *Lycopodium* and *Selaginella* the primary root is rudimentary, and the first functional root is merely one of the endogenous lateral roots developed on the embryonic axis. I shall use the term first root, therefore, to refer to the first functional root.

In *L. complanatum* the first root comes off laterally, almost at right angles to the stem, immediately above the foot region (see *r'*, Pl. XXII, Figs. 1 and 2). The apex was broken off in both sporophytes I examined, but sections nearest the tip show two somewhat bow-shaped groups of tracheides with smaller elements at the periphery, usually more or less collected at the free ends of the bow (*px*, Pl. XXII, Fig. 3); between the two xylem groups lies a strand of phloem occupying the centre of the stele. This agrees in the main with the arrangement described by Bruchmann for the first root of *L. clavatum*, and with that noted by Jones[3] as occurring in a section marked 'first root' which was shown to him as that of *L. clavatum*. Bruchmann calls this a diarch arrangement; Jones says that it may be regarded as diarch or tetrarch. I am inclined to think that this arrangement is more correctly described as diarch.

[1] Treub, Annales de Buitenzorg, vol. iv, p. 133, and Pl. XVIII.

[2] Bruchmann, Über die Prothallien und die Keimpflanzen mehrerer europäischer Lycopodien, 1898, p. 49 and also p. 55.

[3] Jones, loc. cit., p. 26.

Many sections show a few small, apparently protoxylem elements on the outer side of the middle of the bows of xylem elements (see *px*, Pl. XXII, Fig. 3), but much variation exists with regard to the position of these elements. In only one case was the differentiation of the xylem traced in the apical region of a root which showed this two-grouped arrangement of xylem; here xylem elements first appeared at three points, but very soon a row of small elements united two, and the third was extended by the formation of more protoxylem (*px*, Pl. XXII, Fig. 4) into another row, separated from the former by thin-walled elements, some of which, by their larger size and absence of contents, indicate the formation of metaxylem elements (*mx*, Fig. 4). This arrangement appears to be distinctly diarch.

In a rather older sporophyte (Fig. 2) the main root, a little way behind the apex, shows a similar structure to that described above, except that the two xylem groups have united at one end to form a crescent-shaped mass enclosing a central phloem group (Pl. XXII, Fig. 5). The point of union of the two xylem masses is marked by a group of small elements. From this group, a little nearer the base of the root, a somewhat smaller branch passes off to a rootlet, which, though slighter, appears to have originated by dichotomy, and receives a somewhat crescent-shaped mass of xylem (see Fig. 6). Lower down the crescent-shaped mass of xylem in the main root divides again into two groups separated by phloem, and closely resembling the arrangement shown in Fig. 3. It does not seem necessary for these two xylem masses to unite before giving off a branch to a rootlet, as the tracheides may sometimes be seen coming off from one, only, of the xylem masses (see Pl. XXII, Figs. 7 and 8), and sometimes from the two separate groups; in the last case the two smaller groups of xylem which pass into the smaller branch may unite immediately to form a single group.

It will be observed that the branching of the root has been described as dichotomy above. Van Tieghem and Douliot consider that in *Lycopodium* the branches arise immediately behind the apex, but Bruchmann [1], who has studied the branching of the roots in detail, finds that a pure or modified form of dichotomy takes place in the roots of the Lycopodiaceae. He figures longitudinal sections through apices of roots of *L. complanatum* showing dichotomy.

A number of sections made through the roots of *L. complanatum* and *L. clavatum* supported the view that a modified form of dichotomy occurred. It appears that at first one branch of the dichotomy is only slightly developed, while the other continues to bore its way straight down into the soil; gradually, however, the branches get more and more equal, until finally two equal branches occur. These branches appear irregularly arranged on all sides, and they in their turn branch again in a similar manner.

[1] Bruchmann, loc. cit., pp. 73 and 74, Figs. 32, 33, 34, Taf. V.

Near the base of the first root of *L. complanatum* transverse sections show three groups of tracheides with phloem in the centre and extending between the xylem groups (Pl. XXII, Fig. 9). Sometimes two of the three groups unite, cutting up the phloem into a larger and smaller mass, but in both sporophytes of *L. complanatum* examined three separate groups of tracheides occurred in sections nearest the foot. Thus a change from diarch to triarch structure takes place in the first root. A similar change from two to three groups of xylem occurs in *L. clavatum*, where it was traced out and showed that one of the two groups of xylem became elongated laterally and a third group separated off from this. In this species a plantlet at about the same stage of development as the older sporophyte of *L. complanatum* (Pl. XXII, Fig. 2) showed much the same arrangement of xylem and phloem, except that sections nearest the tip showed only a single group of xylem with phloem at one side, i. e. a collateral or monarch arrangement. Thus we may get one, two, and three groups of xylem in the first root.

Immediately surrounding the vascular elements is a layer of cells which is clearly of cortical origin (see Pl. XXII, Figs. 3, 5, 6, and 9, cells marked x). This layer is called the pericambium by Bruchmann; the cells are irregular in shape, and some of the walls show signs of lignification, but in others this feature is absent; sometimes the cells are considerably smaller and more rounded in shape than those of the surrounding cells, in which case they resemble more nearly the parenchymatous cells of the central cylinder. The pericambium is surrounded by an irregular band of cells, two or three cells in thickness, with walls lignified on all sides forming a protective sheath; there is no distinct boundary between these two layers. Outside the protective sheath in the younger parts of the root lie two or three layers of large thin-walled cells with, here and there, small intercellular spaces. At the periphery is the piliferous layer consisting of similar thin-walled cells, some of which have grown out into root-hairs. (In *L. clavatum* the hairs are in groups of three or four, each group being formed from single epidermal cells.) De Bary[1] states that only in *Lycopodium* can special hair-cells be distinguished from other epidermal cells of the root, and goes on to describe the formation of groups of hairs from a part of the lower end of an epidermal cell, which is cut off by an oblique wall as a small cell, and undergoes division into 2–4 cells, each of which grows out into a root-hair. This evidently is the case in *L. clavatum*, but not in *L. complanatum*. Nearer the base of the root, the cells lying next to the protective sheath, to the extent of one or two layers, have very much thickened walls and small lumina (Pl. XXII, Fig. 9), these are surrounded by large thin-walled cells with here and there, just without the thick-walled zone, intercellular spaces,

[1] De Bary, Comparative Anatomy of Phanerogams and Ferns, p. 60.

sometimes of considerable size (see *i.s.*, Pl. XXII, Fig. 9). These air-spaces are apparently produced by the breaking down of the cortical cells; they do not occur throughout the root.

Air-spaces of any considerable size, formed by the breaking down of the cortical cells, are not of frequent occurrence in roots of terrestrial plants. According to Bower[1] air-spaces occur in the internal roots of *L. Selago*, where the outer cortex may be completely separated from the inner cortex by an air-space of schizogenetic origin. He also considers it probable that the outer air-space in Stigmarian rootlets was formed in the same way; and considering the possible relationship of these forms, they may mark the retention of an ancestral character in the Lycopodiaceae from a more aquatic ancestry, though not necessarily from Lepidodendreae.

(*b*) *The second and following roots.* The second and following roots arise endogenously in the apical region of the stem. Bruchmann[2] has studied the origin of the roots in the stems of the Lycopodiaceae, particularly in *L. inundatum*, and found that they arise endogenously in acropetal order from several layers in the cortex, on the ventral side of the stem. He figures a transverse section of the stem of *L. clavatum* showing the origin of the root in these layers. Van Tieghem and Douliot[3], who also studied the origin of the roots of Lycopodiaceae, agreed with Bruchmann with regard to their endogenous origin, but came to a different conclusion as regards the exact layers of the stem which participate in their formation. They consider that the pericycle (pericambium) alone gives rise to the roots.

The young sporophytes of *L. clavatum* show very clearly the endogenous origin of the roots. One of those I examined, the stem of which was about 2 cms. long, showed no less than five rootlets which had not pushed their way out into the soil; they appeared as masses of cells with dense protoplasmic contents and large nuclei in the inner cortex. The exact layers in which they originated could not be traced, as the apical portion of the stem, in which this must be looked for, was destroyed.

Whilst agreeing in the absence of a primary root, the *Lycopodia* differ from *Selaginella* with respect to the origin of the roots, which in the latter, apparently, do not arise directly from the stem in the apical region, but are developed endogenously from exogenous organs (rhizophores), which are found at the base of the stem and at the bifurcations[4]. In this respect the condition in *Selaginella* might be regarded as intermediate between that of *Lycopodium* and *Phylloglossum*, for in the latter the roots arise exogenously.

[1] Bower, On the Structure and Anatomy of the axis of *Lepidostrobus Brownii*. Annals of Botany, vol. vii, 1893, p. 331.

[2] Bruchmann, loc. cit, p. 75, and Pl. IV, Fig. 31.

[3] Van Tieghem and Douliot, Ann. des Sc. Nat., 7e sér., T. viii, p. 553.

[4] Bruchmann, Untersuchungen über *Selaginella spinulosa*, 1897.

The second and following roots of the young sporophytes of *L. complanatum* and *L. clavatum* show a diarch arrangement at their base and throughout the greater part of their length, similar to that shown in Pl. XXII, Fig. 3, while after dichotomy the arrangement may be monarch. The two strands of xylem connect up with two tracheid groups of the stem as shown in Pl. XXII, Fig. 11. The growth of these roots appears to be retarded at first, and, as mentioned above, a whole row of young rootlets which have not grown out beyond the inner layers of the cortex may be seen in the young sporophyte. When favourable conditions for their development arise they push their way out through the cortex, generally more or less at right angles to the stele of the stem. Fig. 10 shows one of these young rootlets in the stem of *L. complanatum* just above the foot region.

The older sporophyte of *L. clavatum* was interesting, as it showed a rootlet which had travelled for some distance down the cortex of the stem (*rt.*, Pl. XXII, Fig. 4) almost parallel with the axis, instead of pushing its way directly to the periphery, and in this respect it resembles the upright *L. Selago* and such forms as *L. serratum*, *L. tetragonum*, *L. reflexum*, *L. squarrosum*, in which the occurrence of rootlets running through the cortex have been recently noted by Jones[1]. Referring to the rootlets in *L. serratum*, Jones[2] says that if the stem is growing quite erect the roots do not pass out, but gradually die out, and that only when the stem is growing obliquely do the roots emerge. The lower part of the stem of the young sporophyte of *L. clavatum* which contained the rootlet running down the cortex was bent at frequent intervals, and possibly the part in which the rootlet occurred was running in an almost upright direction, and this might partly account for its direction. It arose a short distance above the foot, and was the third root, in point of age, proceeding from the stem. The second and fourth roots grew more or less at right angles to the stem. The roots seem to be strongly geotropic, although in some cases other forces, possibly moisture and chemical attraction of other substances in the soil, act more strongly than gravity, and cause them to grow in a different direction. With regard to the internal structure of the rootlet in question, the differentiation of the vascular elements had begun; two curved rows of narrow tracheides were discernible separated by thin-walled cells, some of which by their large size and absence of contents indicated metaxylem. The two groups of xylem elements were connected up with two groups of tracheides in the main axis (see Pl. XXII, Fig. 11), and in this respect resembled the roots running through the cortex of adult forms previously described. In the latter the two xylems usually join up, forming a crescent-shaped mass with small protoxylem elements at the tips of the crescent, but this is not always the case, for in a short piece of stem of *L. dichotomum*

[1] Jones, loc. cit., pp. 22, 23, and 24.

[2] Jones, loc. cit., p. 22.

which I examined, and which contained numerons (as many as seventy) roots in the cortex, in one or two cases the xylems had not joined up to form the crescent-shaped mass on leaving the main stele, but remained separate, resembling those of the rootlet shown in Pl. XXII, Fig. 3; and Jones also noted a few similar cases. Thus the root running through the cortex does not differ materially from the other roots of the young sporophyte as far as the vascular structure is concerned.

(*c*) *Comparison of the vascular structure of the root of mature plants of* L. clavatum, L. complanatum, *and other Lycopodiaceae with that of the young Sporophytes.* In the adult plants of *L. clavatum* and *complanatum* a polyarch vascular bundle is found in the root; the arrangement is radial with the xylem plates often united in the centre. Unequal branching, however, results in the formation of slender branches bearing a much smaller number of xylem groups, and in the finest branches of all a monarch or collateral arrangement occurs. The root of the young sporophyte resembles some of these finer ramifications of the roots of the adult plants in its vascular structure. In *L. Selago* and *L. inundatum*, according to Russow and others, the xylem is diarch and the two masses unite into a single one, which is crescentic in shape, with the phloem in the hollow of the crescent. Jones[1] also found that in the greater number of the roots which run through the stems of such species as *L. Selago*, *L. serratum*, *L. squarrosum*, a similar arrangement occurs, although in a few cases, as mentioned above, the two xylem groups remain separate. In Engler and Prantl's[2] Pflanzenfamilien a figure of the stem of *L. Phlegmaria* is shown, in which there is a rootlet in the cortex, with three groups of xylem united in the centre. This arrangement approaches that occurring at the base of the first root of the young sporophytes of *L. clavatum* and *complanatum*, but differs in having all the xylem groups united in the centre, a condition which is found in the upper part of the stems of the young sporophytes and in slender branches of the adult stem of *L. complanatum.*

According to Campbell[3], who takes Bertrand as his authority, the root of *Phylloglossum* is diarch, but Bower[4], in his paper on *Phylloglossum* read before the Royal Society in 1885, describes and figures a monarch or collateral root. Some specimens of this interesting plant which I had the opportunity of examining, as a number had been sent to Prof. Weiss from New Zealand, showed a monarch root-structure similar to that described by Bower, but in one case a structure very similar to that occurring in *L. Selago* appeared—a crescent-shaped mass of xylem with small elements

[1] Jones, loc. cit., pp. 28 and 29.
[2] Engler and Prantl, Pflanzenfamilien, vol. iv, p. 579, Fig. 365.
[3] Campbell's Mosses and Ferns (new edition), p. 503.
[4] Bower, *Phylloglossum Drummondi.* Phil. Trans. R. Soc., 1885, Part II, p. 674.

at the tips of the horns of the crescent (see Pl. XXII, Fig. 20), possibly indicating that the monarch condition, usually found, is a derived one.

On the whole, the vascular arrangement obtaining in the roots of the young sporophytes resembles more nearly that found in those forms which have roots running through the cortex of the stem, such as *L. Selago*, differing however in the fact that the two xylem groups do not join up immediately behind the point where they join the stele of the stem, as is usually the case with the internal rootlets. It is clear, however, that the monarch condition occurring in certain parts of the root is brought about by the joining-up of the xylem on one side of the stele, and not by the abortion of part of the phloem, as appears to be the case in roots of certain of the Ophioglossaceae, e. g. *O. vulgare*, *O. bulbosum*, *O. reticulatum*, which according to Van Tieghem [1] have diarch arrangement near their insertion, but only one of the lateral phloem groups is developed.

B. *The Foot Region.*

The foot of *L. complanatum* is a rounded structure covered with warty protuberances, which, providing a greater surface, enable it to withdraw more nourishment from the surrounding prothallus. Apparently the foot remains for a considerable time after the disappearance of the prothallus. In the sporophyte shown in Pl. XXII, Fig. 2, where the prothallus has almost entirely disappeared, the cells of the foot are unimpaired even at the periphery, and this, together with the fact that a small branch of the vascular tissue passes into it, would show that there is some possibility that it still acted as an absorbing organ.

Treub [2] considers that the 'embryonic tubercle' in *L. cernuum*, leaving the prothallus and leading a kind of independent existence, represents the foot of the embryo, and that the papillae on the foot of *L. Phlegmaria* correspond to hairs on this embryonic tubercle. The tuber of *Phylloglossum* corresponds in position and structure to the parenchymatous tuber of *L. cernuum*; in both cases the tuber is composed of parenchymatous cells, and the superficial cells may develop root-hairs. Comparing the two, Bower [3] remarks on the absence of a foot in *Phylloglossum*, but says 'it is to be remembered that in this plant the place of the foot is to be taken, physiologically if not morphologically, by the stalk of the young tuber.' The large parenchymatous cells, of which the foot of *L. complanatum* is mainly composed, are strongly pitted. The periphery is well defined in sections stained with haematoxylin, the walls appearing thicker than those of the internal cells on account of the thin layer of protoplasm which lines them. There are deep indentations between each cell, this again affording

[1] Van Tieghem, Traité de Bot., Part II, p. 1394.
[2] Treub, Ann. de Buitenzorg, vol. v, p. 128.
[3] Bower, Phil. Trans., 1885, Part II, p. 675.

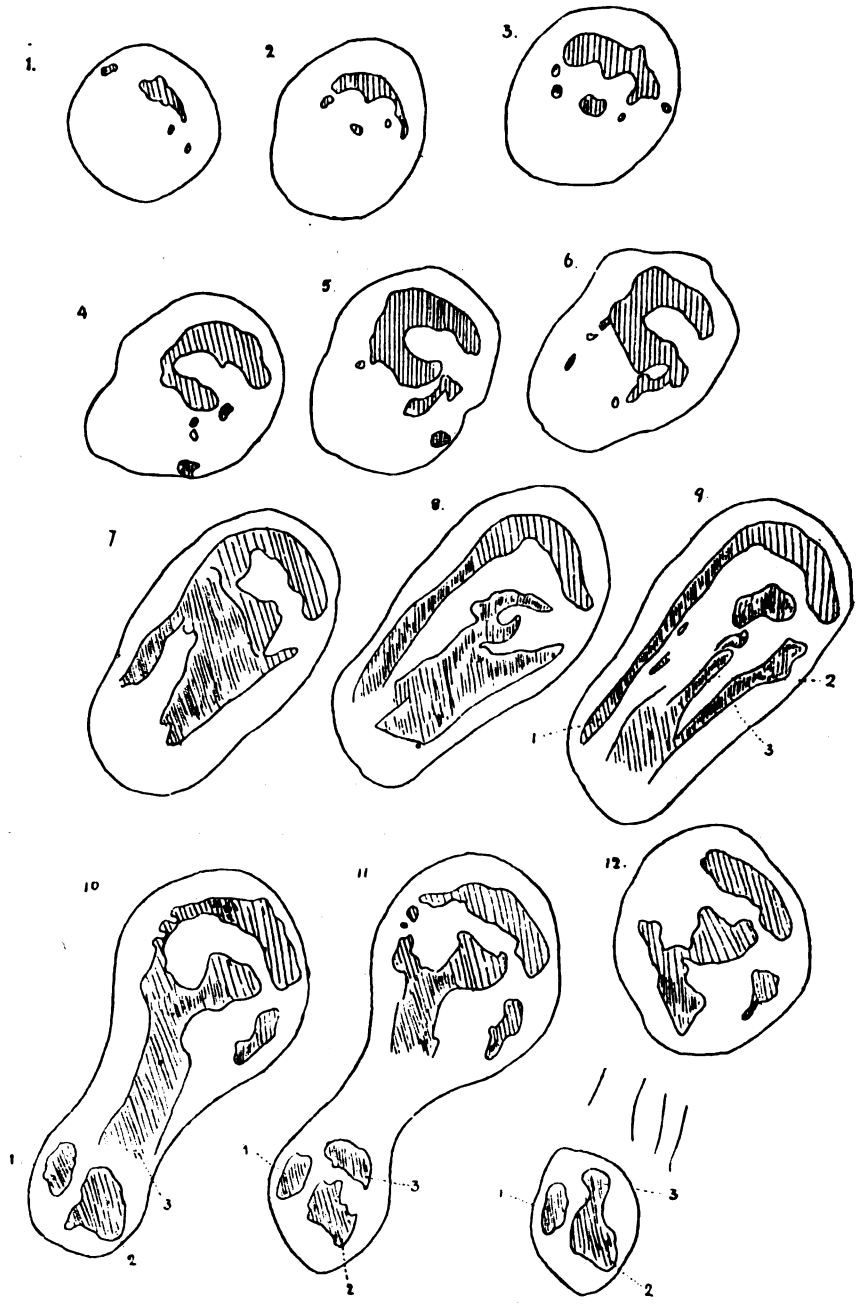


DIAGRAM 1, Figs. 1–12. Showing changes in the vascular arrangement in the foot region and base of the stem of *L. complanatum* (see Pl. XXII, Fig. 2) up to just above the point where the first root is given off, proceeding from below upwards. (Pl. XXII, Fig. 12 shows the first appearance of xylem in the foot.) 1, 2, and 3 in Figs. 9, 10, 11, 12 indicate the xylem strands which belong to the first root; in Fig. 12 two of these have united. Shaded parts represent xylem.

more absorptive surface. Series of transverse sections were made through the foot of both sporophytes of *L. complanatum*. Those of the older sporophyte, which were stained with iodine green and Bismarck brown, showed more clearly the rather thin-walled lignified cells which are connected with the 'protective sheath' of the stem and root than did the sections of the foot of the younger sporophyte, which were stained with haematoxylin and saffranin. Proceeding upwards from the base of the foot, the first appearance of lignification of the cell walls is shown in the centre of the foot, where about half a dozen cells took the iodine green stain. The area of this patch increases gradually, the cells appearing more or less uniform, with pitted lignified walls, until about twenty to thirty cells are included; then the lignification of the walls of the central cells becomes less and less until a distinct core of small thin-walled unlignified cells appear in the centre. A little higher up a tracheide appears a little to one side of this central patch (see *t*, Pl. XXII, Fig. 12), and soon more tracheides are formed, until a bow-shaped group parallel with the periphery occurs (see Pl. XXII, Fig. 13). Diagram 1 (Figs. 1–6) shows the further changes in the arrangement of the xylem of this vascular strand in the foot region of the older sporophyte of *L. complanatum*. It will be seen that three or four small groups of tracheides make their appearance in addition to the main group, but they vary in position, size, and number in the two sporophytes. There is, however, a tendency towards concentration of the xylems to form a somewhat crescent or hook-shaped mass of xylem (see Diagram 1, Figs. 4, 5, 6, 7, 8, and 9); from this, three strands, 1, 2, 3 (Figs. 9, 10, and 11), pass off to the first root, and in the case of the older sporophyte run in an upward direction, making an acute angle with the stele of the stem, and then turn at right angles to the main stem (see Diagram 1, Figs. 3–12). In Fig. 12 it will be seen that two of the three strands have fused in the root.

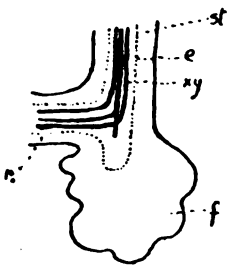


DIAGRAM 2. A diagrammatic sketch of a longitudinal section through the foot region of the young sporophyte of *L. complanatum* (see Pl. XXII, Fig. 1) showing the course of the vascular strands. *st*, base of stem; *r*, root; *f*, foot; *e*, protective sheath; *xy*, xylem strands.

In the younger sporophyte of *L. complanatum* the three xylem strands belonging to the root do not take an upward course, but pass off immediately at right angles to the stem (see Diagram 2).

In the young sporophytes of *L. clavatum* no vascular strand was observed in the foot. The smaller one showed two groups of xylem with a single strand of phloem throughout the root, but immediately above the foot, large vessels connected the two groups, separating the phloem into two portions, and very soon three groups of xylem separated out, the phloem between joining up and forming a three-rayed star. The older

sporophyte showed three groups of xylem at the base of the root, but throughout the greater part of the foot region two of the groups united, but separated out again above the foot, forming an arrangement similar to that at the base of the root.

C. *Stem.*

(*a*) *General structure.* Transverse sections through the stem (Pl. XXII, Figs. 11, 14, 15) show that the centre is occupied by a single stele, which remains undivided except where branching occurs, and, as Mr. C. E. Jones [1] points out, there is no evidence from the structure of the young sporophyte in favour of the view put forward by Strasburger that the central cylinder of *Lycopodium* may be regarded as gamostelic, in the ordinary sense of the word. The xylem consists of narrow spiral or annular protoxylem elements, which generally form bow-shaped groups. The metaxylem shows transitions from pitted to scalariform elements. In the narrower elements the pits appear rounded, and extend across the breadth of the tracheides, in the wider elements they are elliptical in shape, and appear as bordered pits, whilst in the widest elements they are much elongated in a radial direction, forming scalariform tracheides, or they remain small, several rows appearing on the longitudinal walls (see Pl. XXII, Fig. 16). The metaxylem elements are formed centripetally, and generally connect two or more of the groups of protoxylem. Near the foot region these groups may remain unconnected, the phloem occupying the central portion of the stele, as is usually the case with the root whether diarch or triarch, but at the point where the first root comes off a fusion usually occurs.

Pl. XXII, Fig. 14 shows a transverse section of the lower part of the young sporophyte figured in Pl. XXII, Fig. 2. Here it will be seen that the central mass of metaxylem has become quite separated off; this, however, is not the usual condition; generally, the central metaxylem is connected with one or more protoxylem groups (see Diagram 1, Fig. 12).

Alternating with the xylem groups are the phloem elements, consisting of sieve-tubes and parenchyma. The sieve-tubes are long and narrow; the walls appear to be covered with very finely pitted areas in older parts of the mature plant of *L. complanatum*. In this they agree with larger forms of *Lycopodium*, such as *L. Hippuris*, where the walls of the sieve-tubes have apparently sieve-plates, the pitted areas of which lie so close together that a reticulate appearance is given (see Pl. XXII, Fig. 21).

With regard to the contents of the sieve-tubes, negative results were obtained on testing for leptomin with guaiacum and hydrogen peroxide and with alpha naphtha dissolved in water and hydrogen peroxide; this may,

[1] Jones, loc. cit., pp. 17, 27.

however, be due to an insufficient quantity of this substance being present to be detected by these tests, as the sieve-tubes are very narrow.

The cells immediately surrounding the protoxylem are of cortical origin in the lower region of the stem, and also in the upper parts in those plantlets which have not appeared above the ground. The following regions can be distinguished external to the stele (see Pl. XXII, Fig. 14): (1) an irregular ring (*pc*) of thin-walled, fairly large cells (pericambium), which abut on the xylem and phloem regions. Some parts of the walls of these cells show signs of lignification, so that it is often difficult to draw a distinct boundary between this and (2) the protective sheath (*en*), which immediately surrounds it, and which also consists of an irregular band of cells, one, two and occasionally three cells in thickness, the walls of which are not thickened but strongly lignified. Outside the protective sheath are (3) thick-walled cortical elements (*ic*), closely packed cells, the walls of which show lignification at the corners in the neighbourhood of the protective sheath-cells. Proceeding outwards, the cells become larger and thinner-walled, gradually merging into (4) a much more loosely arranged layer of cells (*nc*), in which large intercellular spaces occur. It is the presence of these large intercellular spaces in the outer cortex, and also the greater differentiation of cells forming the cortex and epidermis, which distinguish the basal region of the stem from that of the root in the neighbourhood of the foot, where the stelar structure is very similar. Bounding the cortex externally is (5) the epidermis (*ep*), the cells of which vary in size, especially in the underground parts; their outer walls are thickened, and in the aerial parts numerous stomata occur.

(*b*) *Changes occurring in the vascular structure.* The arrangement of the xylem and phloem elements is subject to many changes, especially in the lower region of the stem, where the various obstacles met with in the soil have resulted in the stem taking a very irregular course. The strands of xylem are constantly uniting, then separating, or by extension of the protoxylem elements at the periphery of the stele a further subdivision may take place. There is, however, in the young sporophyte a tendency towards the formation of a solid core of xylem in the centre of the stele, and in the upper parts of the stem a more constant arrangement is maintained. Here a tri- or tetrarch condition occurs, large metaxylem elements in the centre uniting two groups of protoxylem, sometimes a third, and occasionally a fourth group. When all the groups are united the triarch condition resembles that occurring in the smaller branches of *Psilotum* and in young stems of *Sphenophyllum* in which secondary thickening has not begun.

Diagram 3, Figs. 1–10, shows the changes which occur in the arrangement of xylem and phloem in the younger sporophyte of *L. complanatum*, proceeding upwards from the point where the stele of the first root joins

that of the stem, as seen in transverse sections. The xylem strands appear to have united in Fig. 2, but immediately above separate again, and an

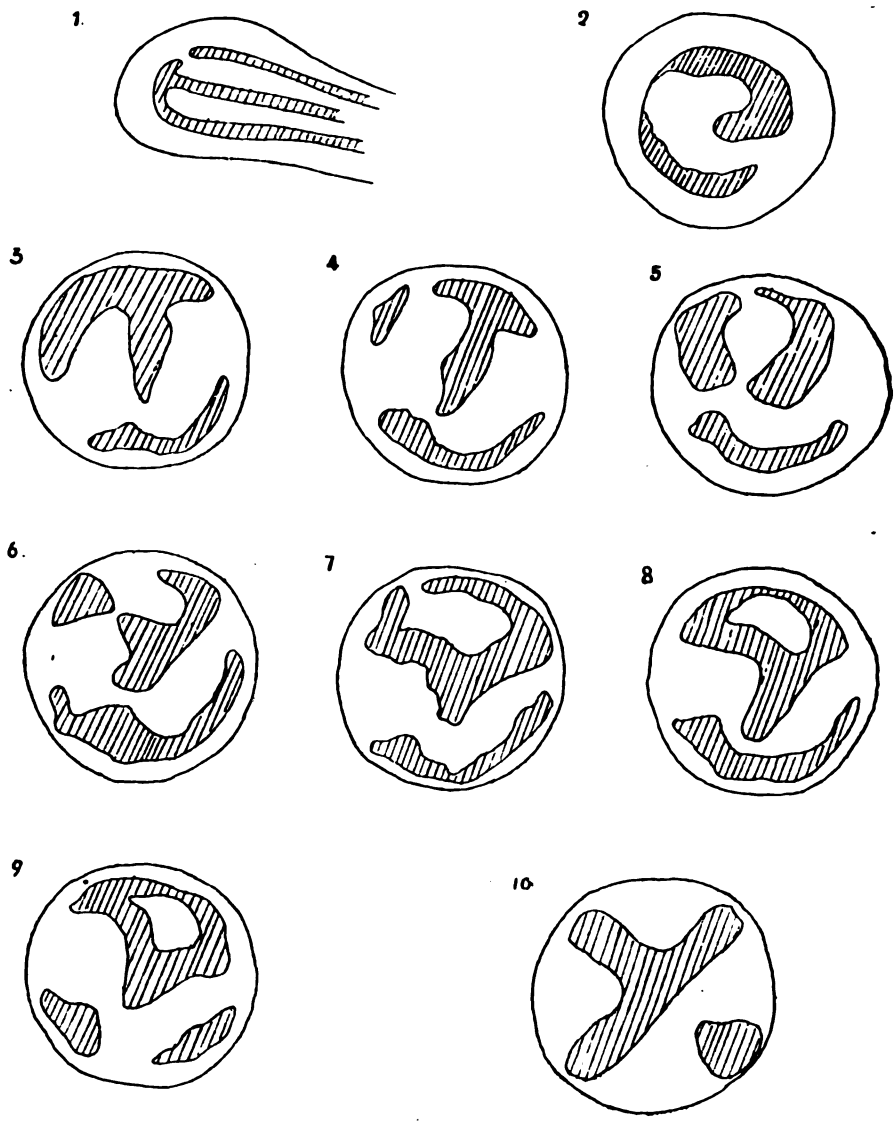


DIAGRAM 3, Figs. 1–10. Showing changes which take place in the vascular arrangement of the stem of the young sporophyte of *L. complanatum* (see Pl. XXII, Fig. 1), proceeding from the base upwards. Fig. 1 shows three xylem strands passing off to the first root. Shaded parts represent xylem.

arrangement similar to that occurring in the root is found (Diagram 3, Figs. 4 and 5; and for root, Pl. XXII, Fig. 9).

In the older sporophyte of *L. complanatum* a complication was brought about in the stelar arrangement, due to the fact that dichotomy had taken place very early; one of the branches had been arrested in its growth, forming what Bruchmann [1] describes as a pseudo-adventitious bud, while the other was continued as the main stem and had again dichotomized several times. In the pseudo-adventitious bud, although this was only visible as a slight swelling near the foot, a root had already begun to grow out (Fig. 15). The apical growth of the branch had been much delayed, yet a stimulus to rapid growth had evidently been given, for the apex had just dichotomized, and the root was pushing through the outer layers of the cortex.

The changes in the arrangement of the xylem and phloem in the main stem of this second sporophyte were very rapid. In Diagram 1, Figs. 11 and 12, and Diagram 4, Figs. 1–23, some of the changes which occur in the first 2 mm. of the stem region above the point where the first root comes off are shown. It will be seen that sometimes the xylem groups all unite, forming variously shaped masses (see Diagram 4, Figs. 10, 11, 16, 20, 21); sometimes islands of phloem are formed by the closing round of the xylem (see Diagram 4, Figs. 10, 16, 19, and 20), or the phloem may remain external to the xylem surrounding it, and forming numerous bays or cutting off smaller or larger portions, of various shapes (see Diagram 4, Figs. 18, 19, 22, 23). There is, however, a tendency to the formation of a central mass of metaxylem. Diagram 4, Fig. 23, shows a section in which a central mass of metaxylem is separated off from three groups of smaller elements. As a rule, however, the central mass remains connected with at least one of the protoxylem groups.

In the sporophytes of *L. clavatum* there was less complication in the course of the xylem strand throughout the stem. A short distance above the point where the first root came off there were three separate groups of xylem, with phloem in the centre and extending between the groups; then one or two of the groups of xylem united at the periphery, apparently by extension of the protoxylem elements, and immediately after four groups separated out. Higher up two of the groups united again, by extension of protoxylem elements, and at the same time by centripetal development of metaxylem either all three groups united in the centre or one remained separate. The older sporophyte showed variations from the arrangement described above, in some parts of the upper portion of the stem, the protoxylem elements extending towards the centre in such a manner that a very irregular arrangement occurred, but on the whole the tendency was for the arrangement to become more uniform, producing a radial arrangement with the number of rays increased in the stouter portions of the stem and where dichotomy was about to take place.

[1] Bruchmann, loc. cit., p. 70.

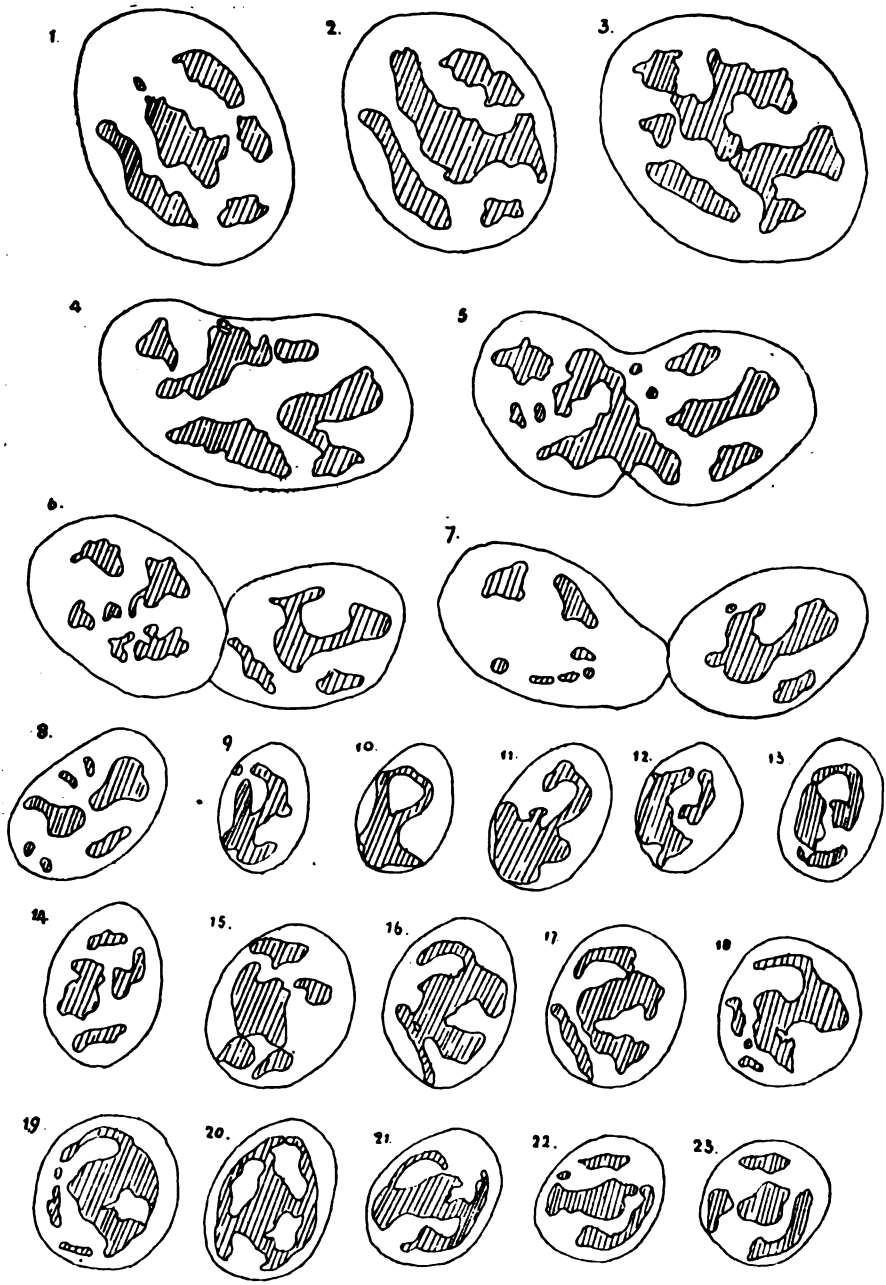


DIAGRAM 4. Showing some of the changes which take place in the vascular arrangement at the base of the stem of the young sporophyte of *L. complanatum* (see Pl. XXII, Fig. 2) above the point where the first root comes off. This continues the series of sections shown in Diagram 1. Dichotomy is illustrated by the first seven figs.; the remaining, Figs. 8–23, show only the stele of the main branch, i. e. the right-hand one in Figs. 6 and 7. (Pl. XXII, Fig. 15, represents a stage occurring between Diagram, Figs. 9 and 10.) Shaded parts represent xylem.

In the upper part of the older sporophyte of *L. complanatum* a tendency towards the banded arrangement is shown; the xylem mass, which here is six-rayed, has become separated into two by a band of phloem. In this region also and in the older parts of *L. clavatum* a layer of cells occurs between the protoxylem and the 'pericambium,' which resemble the phloem-cells in general appearance and in the staining properties of the walls. Van Tieghem calls the band of cells, one to several layers thick, between the endodermis (protective sheath) and the xylem the pericycle, and regards it as part of the central cylinder. Strasburger and Bruchmann [1], on the other hand, consider that this pericycle has the same origin as the cortex. I find that in the lower part of the stem the protoxylem elements do lie next to cells of cortical origin, occasionally next to the protective sheath itself, but that in the upper part of the older sporophyte there is a layer of cells which belongs to the central cylinder lying between the protoxylem and the cells of cortical origin, and probably representing phloem-cells. This latter condition agrees with that of the mature stem, where there is generally a ring of thin-walled cells lying between the protoxylems and the endodermis which resemble the cells surrounding the sieve-tubes between the xylem rays. The view that this layer outside the xylem represents phloem does not seem inconsistent when one considers the irregular course which the xylem strands take through the stem of the young sporophyte, and the fact that the leaf-traces play an important part in the constitution of the central stele, except in the lower part of the young sporophytes.

According to Campbell [2] the complete stem of *Lycopodium* possesses two systems of vascular bundles, the strictly cauline central cylinder and the system of common bundles formed by the leaf-traces.

In the lower part of the young sporophyte the central stele is strictly cauline. The first indication of anything approaching a leaf-trace is the spiral marking of some of the cortical cells which are in connexion with the base of the scale. In a slightly more advanced stage the leaf-trace is represented by exceedingly narrow tracheides which leave the main stele at the edge of one of the protoxylems. Higher up, where the leaves have reached the light and are better developed, the structure of the leaf-trace is more complicated, the number of elements increasing as it passes through the cortex. The leaf-trace leaves the main stele at the end of the xylem plates and takes an upward course through the cortex, making an acute angle with the central stele of the stem, while near the periphery it takes a more horizontal course. A section taken just above the point where it leaves the stele of the stem shows a well-defined mass of xylem consisting of tracheides which in transverse section appear to be full of granules owing

[1] Bruchmann, loc. cit., p. 55.

[2] Campbell, Mosses and Ferns (new edition), p. 495.

to the ingrowth of the thickened part of the wall [1] (Pl. XXII, Fig. 17), these are surrounded by elongated parenchymatous cells with large nuclei, which connect up with the ring of parenchyma which surrounds the main stele and which appears to be of the nature of phloem, although true sieve-tubes have not been made out in the leaf-trace. Russow [2] states that the leaf-trace in *Lycopodium* consists of a few spiral and reticulate cells, which are surrounded by companion cells and a few protophloem elements. In the young sporophytes I examined the elements forming the leaf-trace were so small that I was unable to distinguish sieve-plates in the elongated cells surrounding the xylem.

When the leaf-trace has passed beyond the endodermis it can be seen that the number of elements surrounding the xylem has increased, and that some of the walls of the 'phloem' cells have become thickened, so that it is difficult to distinguish between the xylem and the remaining elements in transverse sections, as the walls of the xylem elements contain little or no lignin. The increase in the number of elements forming the leaf-trace is not peculiar to this species. It is well marked in *L. Phlegmaria*, which possesses very large leaf-traces, and here it can be clearly seen that the leaf-trace is surrounded by a protective sheath of cells resembling those surrounding the central cylinder of the stem, and that they have divided by walls parallel to the periphery of the bundle.

Bower [3] found that, amongst the species of *Lycopodium* he examined, the leaf-trace of *L. Phlegmaria* most resembled that of *Lepidostrobus Brownii*, both with regard to the small amount of tissue referable to phloem and the division of the outer cells by walls parallel with the limits of the bundle. *Tmesipteris* also shows a resemblance in these respects. The leaf-trace of *Lepidostrobus Brownii* is, however, collateral, and in recent Lycopods it appears to be concentric.

(*c*) *Comparison of the stem of the young sporophyte with that of the mature plant.* Comparing the structure of the stem of the young sporophyte of *L. complanatum* with that of the mature plant, it will be seen that the stele in the upper part of the branches of the latter closely resembles that of the upper part of the young sporophyte in there being 3–5 protoxylem groups radially arranged and connected by metaxylem in the centre, with usually one or more groups separated off from the rest and surrounded by a layer of parenchyma. The chief differences lie in the irregular and rapidly changing arrangement of xylem and phloem in the basal portion of the stem of the young sporophyte, and in the shape of the stem—that of the young

[1] De Bary describes somewhat similar tracheides, which he calls 'trachae trabeculatae,' as occurring at the corners of the vascular bundle of the stems of stronger species of *Lycopodium*, and in the margin of the vascular bundles of the leaves of *Juniperus*. Compar. Anatomy of Phanerogams and Ferns, pp. 163, 164, Fig. 62.

[2] Russow, Mém. de l'Académie Impériale des Sc. de St-Pétersbourg, 7e Sér., vol. xix.

[3] Bower, On Structure of *Lepidostrobus Brownii*. Ann. of Botany, vii, 1893, p. 340.

sporophyte being more or less round in transverse section, while that of the mature plant is like a flattened triangle, owing to the shape of the leaves and their fusion with the stem. They are fused in such a manner that the chief part of the cortex, consisting of radially elongated assimilating cells, belongs equally to the leaves and stem, and this distinguishes the cortical tissues from those of the young plantlet, in which the assimilating cortical cells run in a vertical direction. Another difference lies in the distribution of the stomata; these occur all round the stem of the young sporophyte, but are confined to the flattened ventral side of the mature stem. The creeping underground part of the stem of the mature sporophyte naturally shows greater differences. Here the stem is much more bulky and circular in transverse section; the number of protoxylem groups has increased to a dozen or more, and these are connected in twos or threes by metaxylem in such a manner that the well-known banded arrangement is produced. The parenchymatous zone round the xylems is more pronounced, and the walls of the cells of the inner cortical zone have thickened so much that only very small lumina are left. The presence of large intercellular spaces in the surrounding cortical layers and stomata in the epidermis, together with the fact that small leaves provided with vascular bundles occur at intervals, indicate an ancestral condition in which this part of the stem was also aerial. Evidence drawn from the study of the young sporophyte seems to point to the view that the radial arrangement of xylem and phloem occurring in the upright form of *Lycopodium* is the more primitive condition, and this is supported by evidence from fossil forms of the Lycopodiales.

(*d*) *Branching of the Stem.* The branching of the stem, as in the roots, is dichotomous. This may begin very early, but in most cases the growth of one of the branches is stronger than that of the other, and so sections taken immediately below the dichotomy show one of the two steles smaller than the other. In the case of the pseudo-adventitious bud, the growth of one of the branches of the dichotomy is arrested for a considerable period, and it remains as a bud protected by a few leaves until favourable circumstances arise for the continuation of its growth, and then not only apical growth occurs, but a root may be produced at the base, *rt.* Pl. XXII, Fig. 15, so that if separated off from the main plant it could lead an independent existence.

(*e*) *The Apex of the Stem.* Longitudinal sections were made through the apex of the sporophyte shown in Pl. XXII, Fig. 2. This had dichotomized, and one of the branches had grown rather longer than the other. The shorter branch had just dichotomized, and showed at the apex two smaller groups of cells forming somewhat flattened cone-shaped structures about equal in size. A definite apical cell could not be made out; the actual apex appeared to be occupied by several large cells equal in size, from which,

first by periclinal walls and then anticlinal, the cells which give rise to the various tissues of the stem are cut off.

D. *The Leaves.*

The first leaves of the sporophyte are of very simple structure. They are merely scales consisting of small cells, rounded near the apex and becoming more elongated when they join on to the stem.

In the smaller sporophyte of *L. complanatum* shown in Pl. XXII, Fig. 1, although a number of these scales were produced, there was no trace of a vascular bundle in any of them. They were not more than three cells in thickness at the widest part, and the central row appeared in no way different from the outer ones. A longitudinal section near the apex of the shoot showed a few very long, spirally marked cells, running from the stele of the stem to the base of the scale-leaf.

In the older sporophyte (Pl. XXII, Fig. 2) the differentiation of the leaf-tissues had begun earlier; probably this was connected with the formation of a pseudo-adventitious bud. The stem had evidently dichotomized above the foot. In a series of sections cut through this region the first leaf appears as a scale, without any vascular bundle, opposite to the point where the first root leaves the stem. The second leaf appears slightly higher up, about a quarter of the distance round the stem. I could not verify the description of Bruchmann[1], according to which the first two leaves are opposite, as in *Selaginella*. The same series of sections shows dichotomy of the stele completed, and the third leaf appears on the side of the arrested branch. About the same time a leaf cut longitudinally shows a very simple vascular strand consisting of a few very narrow tracheides, the continuation of which can be traced as far as the protective sheath of the central cylinder. Higher up, the differentiation of the tissues of the leaves is more marked, but they do not attain a great degree of complexity in the young sporophyte.

The vascular bundle of the leaf extends about four-fifths of the length of the leaf; it is made up of a few spiral or reticulate tracheides, which are, in the lower part of the leaf, surrounded by narrow elongated cells with thickened walls, of the nature of tubes containing granules, and in some cases nuclei (*ph*, Pl. XXII, Fig. 18), and of parenchymatous cells, some of which contain chlorophyll corpuscles.

Erikson[2], in his paper on the anatomy of the leaves of the Lycopodiaceae, notes that in many forms the outer bast cells contain chlorophyll, but does not mention *L. complanatum* in this connexion. He distinguishes four types of bast as occurring in the leaves of the Lycopodiaceae[3]:—(1) thick-walled and lignified bast, as in *L. annotinum* and *clavatum*; (2) thick-walled

[1] Bruchmann, loc. cit., pp. 53, 68.

[2] Erikson, Arbeten från Lunds Botaniska Institution, 1892.

[3] Erikson, loc. cit., p. 35.

and unlignified as in *L. rufescens*; (3) singularly thin-walled bast, as in *L. guidoides;* (4) bast with thin walls in the interior and thick-walled on the exterior, as in *L. tetragonum.* He includes *L. complanatum* in the same group as *L. annotinum, L. clavatum, L. magellanicum,* and *L. alpinum,* forms which are characterized by their very woody bast, slanting palisade cells, and strongly developed stomata. I was unable to detect lignification in the walls of the bast-cells of *L. complanatum,* but they appear thickened.

A definite endodermis was not distinguishable in the leaves of *L. complanatum,* and the bundle elements seemed to gradually merge into the more loosely and irregularly arranged parenchyma forming the mesophyll. Stomata occur on both upper and under sides of the leaf of the young sporophyte.

The leaves arise very close together, and are irregularly arranged in four rows round the stem. They begin as small projections formed by two or three of the epidermal cells near the apex growing in a radial direction, and rapidly dividing by periclinal and anticlinal walls. In the very early stage an apical cell appears, and from this cells are cut off for the upper and under side of the leaf, while periclinal walls cut off cells for the central tissue; at the same time there is active division taking place at the base of the leaf, and while, after a time, the apical cell ceases to divide, intercalary growth at the base continues until the leaf has reached its full length. At a very early stage the cells bordering the apex begin to elongate, forming the long tapering point characteristic of the mature leaves, and the differentiation of the vascular strand soon begins. In the stem the tracheides of the leaf-trace are the first to appear; they occur at the periphery of the central stele and then run obliquely upwards through the cortex of the stem, extending towards the tip of the leaf, but never quite so far as the actual apex.

Stomata are developed on both sides of the leaf, and, as the leaf grows, air-spaces become more numerous, especially in the lower region, and are connected with the air-spaces of the stem, the cortex of the stem passing insensibly into the bases of the leaves.

The leaves of the young sporophytes of *L. complanatum* show considerable differences from those of the aerial part of the mature plants, both in arrangement and general structure.

In the young sporophytes they are arranged in four rows in an irregular spiral manner, often two leaves occurring opposite or almost opposite each other, but at the base considerable internodes generally intervene. They are all linear lanceolate in form, the lower half of the leaf closely adhering to the stem. In the mature plant the leaves are crowded together, and fused with the stem in such a manner that one cannot tell where the leaf ends and the stem begins; only a small portion of the leaf is free from the stem. They are arranged very regularly in four rows

round the stem, and owing to the dorsiventrality of the latter, three different kinds must be distinguished, exposed to different degrees of light, moisture, &c., namely, dorsal, ventral, and lateral leaves. The ventral leaves are narrow, and gradually taper to a point; a very small portion of the leaf is free from the stem, the remaining part is fused with it in such a manner that the division between the two is at once lost sight of, and the whole ventral side of the shoot appears slightly concave.

The dorsal leaves are also long and narrow, but the tapering near the apex is more sudden than in the ventral leaves, and the portion of the leaf fused with the stem projects from the surface of the shoot until it is hidden by the free part of the next leaf below.

The lateral leaves are arranged in pairs almost opposite to each other; they are keeled, and only a very small portion of the leaf is free from the stem. The free portion tapers gradually to a point, and is triangular in transverse section; the fused portion is extensive, and appears to form the chief assimilating organ.

Comparing the distribution of stomata on the leaves of the mature plant and those of the young sporophyte, it appears that whereas on the latter the stomata are scattered in about equal numbers on both surfaces, in the former their distribution is restricted to certain areas. On the outer side of the ventral leaves stomata occur only on the basal portion, while on the inner side they are scattered over the surface, almost extending to the apex. On the outer side of the dorsal leaves stomata are absent, while on the inner side they are restricted to a small area a short distance below the tip. In the case of the lateral leaves, three surfaces must be considered —upper, under, and inner, the two former representing the outer surface of the leaf. On the under side numerous stomata occur near the base of the free portion, especially on that part which lies nearer the stem, and on the fused portion except near the outer edge. Stomata are absent on the upper side, but are present on the inner surface of the leaf. The vascular bundle does not appear to differ materially from that of the aerial leaves of the young sporophyte described above.

Summary.

The main results obtained during this investigation may be summarized as follows:—

1. The first root of the young sporophytes of *L. complanatum* and *L. clavatum* may show a monarch, diarch, and triarch arrangement in the same plantlet; the xylem groups are usually separated by phloem, which occupies the centre of the stele and extends between the xylem groups to its periphery.

2. The second and following roots show a diarch arrangement, except the fine ramifications, which are monarch.

3. The roots arise endogenously near the apex of the stem, except the first root, which, according to Bruchmann, arises endogenously from the base of the 'hypocotyl.' The growth of the roots formed at the apex remains arrested for a time, and then they either grow out at right angles to the stem or they may take a downward course through the cortex of the stem.

4. The branching of the roots appears to be a form of dichotomy, but one branchlet is generally less vigorous than the other, and receives a smaller strand of vascular tissue.

5. In *L. complanatum* a short strand from the main stele passes into the foot, and the appearance of the peripheral cells of the latter is such that the possibility that it may still act as an absorptive organ after the disappearance of the prothallus is not excluded.

6. The arrangement of the vascular tissues at the base of the stem shows much irregularity. The xylem strands appear to be continuous with those of the first root, and an arrangement very similar to that occurring at the base of the root may be seen immediately above the point where the root comes off. At this point a fusion of two or of all three xylem strands takes place.

7. The xylem strands take a very irregular course, fusing with one another and subdividing in the lower part of the stem, probably due to the frequent bending in this region.

8. The upper part of the stem shows a tri- or tetrarch arrangement of xylem with central metaxylem, generally connected with two or more groups of protoxylem.

9. The apex of the stem is occupied by several large actively dividing cells.

10. Branching of the stem takes place by dichotomy, but the growth of one of the branches is generally stronger than that of the other; the retardation of one of the branches may result in the formation of a pseudo-adventitious bud in the case of *L. complanatum*.

11. The first leaves are scale-like, without vascular bundles, and arranged in an irregular spiral round the stem. The later leaves have a vascular strand, which consists of a few very narrow tracheides surrounded by a more or less thick-walled bast.

In conclusion, I should like to add that this investigation was undertaken at the suggestion of Professor Weiss, to whom my best thanks are due for the valuable suggestions and advice which he has given me throughout. I also wish to express my thanks to Dr. Bruchmann for his readiness in agreeing to the publication of this investigation, made upon material generously supplied by him and intended primarily for museum purposes.

EXPLANATION OF PLATE XXII.

Illustrating Miss Wigglesworth's Paper on *Lycopodium*.

Figs. 1 and 2. *L. complanatum*. Young sporophytes. r^1 = first root, r^2 = second root, *ft* = foot, *pa* = pseudo-adventitious bud, l^1 and l^2 = first and second scale-leaves. Fig. 1 × 1, Fig. 2 × 1½.

Fig. 3. *L. complanatum*. Transverse section through root showing two groups of xylem. *en* = protective sheath, *pc* = pericambium, *px* = protoxylem, *ph* = phloem. × 150.

Fig. 4. *L. clavatum*. Portion of a transverse section through the lower part of stem showing a diarch rootlet in the cortex. *rt* = rootlet, *px* = protoxylem, *mx* = metaxylem, *ph* = phloem, *ep* = epidermis of stem. × 164.

Fig. 5. *L. complanatum*. Transverse section through central region of rootlet showing two xylem groups fused, forming a crescent-shaped mass. Lettering as in Fig. 3. × 200.

Figs. 6, 7, 8. *L. complanatum*. Transverse sections through central region of rootlet showing branching. In Fig. 6 the xylem of smaller branch separates off from two groups of xylem which have previously fused to form a crescent-shaped mass; in Fig. 7 it comes off from only one of the xylem groups; Fig. 8 shows the xylems of the two branches completely separated. Lettering as in Fig. 3. × 200.

Fig. 9. *L. complanatum*. Portion of transverse section through basal region of first root showing three groups of xylem. *ep* = epidermis, *c* = cortex, *i.s.* = intercellular space, *pc* = pericambium, *en* = protective sheath, *px* = protoxylem, *ph* = phloem. × 300.

Fig. 10. *L. complanatum*. Portion of transverse section through lower region of stem showing young rootlet. *ep* = epidermis of stem, *i.s.* = intercellular space, *c* = cortex, *en* = protective sheath. × 109.

Fig. 11. *L. clavatum*. Transverse section through stem at a slightly higher level than that shown in Fig. 4 showing connexion of the rootlet stele with that of the stem. *rt* = rootlet, *st* = outer tissues of stem. × 90.

Fig. 12. *L. complanatum*. Transverse section through foot region. *p* = remains of prothallus, *en* = protective sheath, *t* = tracheide. × 71.

Fig. 13. *L. complanatum*. Transverse section through central region of foot at a slightly higher level than that shown in Fig. 12. Lettering the same as in Fig. 12. × 150.

Fig. 14. *L. complanatum*. Transverse section through lower region of stem showing isolated central metaxylem and three groups of protoxylem at the periphery of the stele. A leaf is cut longitudinally and a portion of the leaf-trace is shown. *lt* = leaf-trace, *l* = leaf, *pc* = pericambium, *en* = protective sheath, *ic* = inner cortex, *mc* = middle cortex, *ep* = epidermis. × 82.

Fig. 15. *L. complanatum*. Transverse section through stem and pseudo-adventitious bud (*pa* in Fig. 2) showing main branch of stem (*a*) and retarded branch (*b*) which has produced a root (*rt*). *l* = leaf. × 71.

Fig. 16. *L. complanatum*. Xylem elements of stem in longitudinal section.

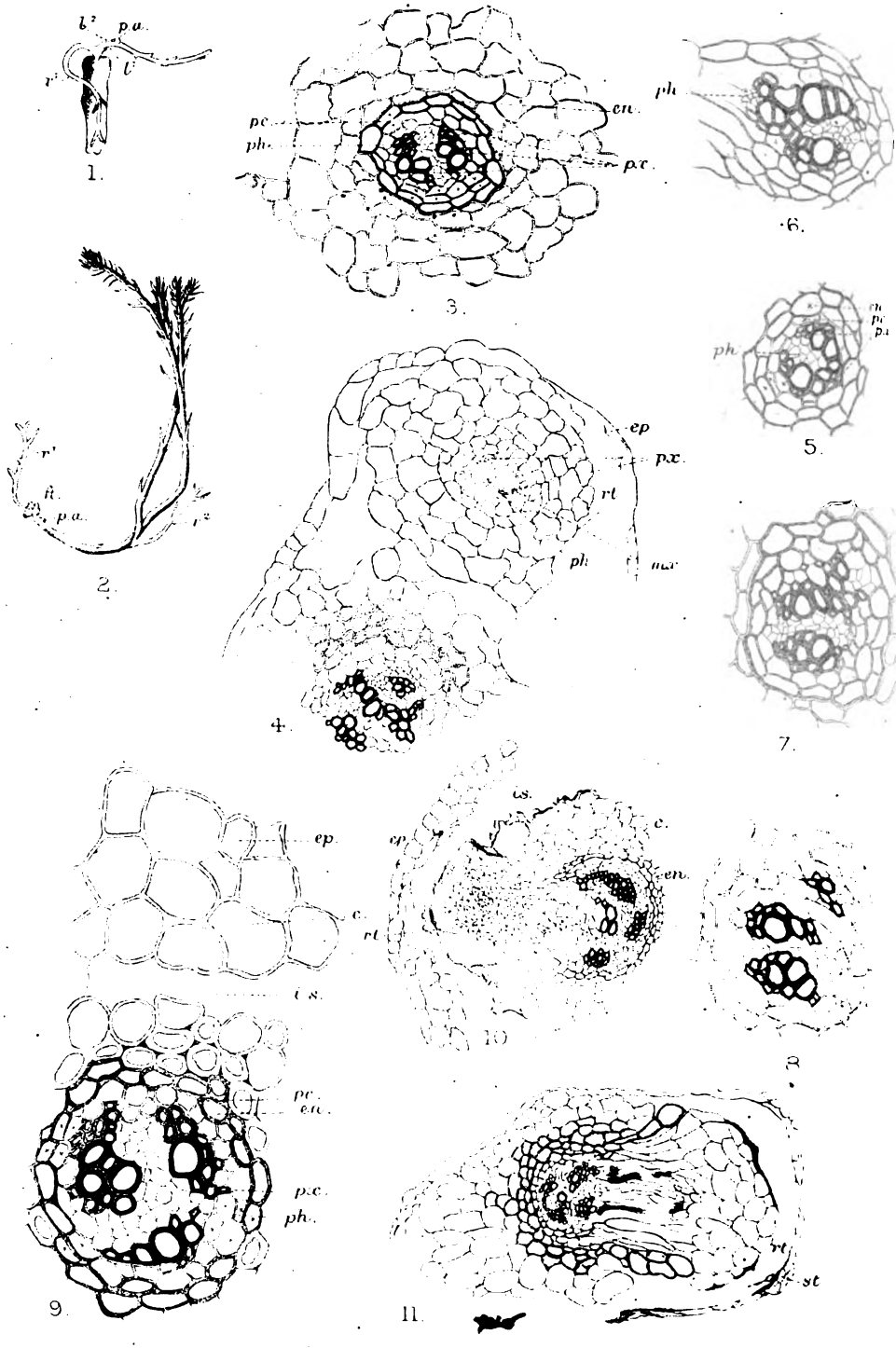
Fig. 17 *a*, *b*, *c*. *L. complanatum*. Successive transverse sections through the peripheral portion of one of the xylem rays and the surrounding tissues in the upper part of the stem showing outgoing leaf-trace, the dotted cells represent the curiously thickened tracheides of the leaf-trace. In 17 *c*, where the leaf-trace tracheides have become separated from the main xylem mass, they are surrounded by cells with large nuclei which apparently represent phloem. *en* = protective sheath, *lt* = leaf-trace, *ph* = phloem, *px* = protoxylem. × 147.

Fig. 18. *L. complanatum*. Part of a longitudinal section through a leaf on the upper part of the stem showing thick-walled phloem. *ep* = epidermis, *m* = mesophyll, *i.s.* = intercellular space. *t* = tracheide, *ph* = phloem. × 247.

Fig. 19. *L. complanatum*. Transverse section through the central tissues of leaf from upper part of the stem. The vascular bundle is represented by thick-walled cells, the outer ones of which contain protoplasm and represent phloem (*ph*). × 187.

Fig. 20. *Phylloglossum Drummondi*. Transverse section through stele of a root. *en* = endodermis, *px* = protoxylem, *ph* = phloem.

Fig. 21. *Lycopodium Hippuris*. Sieve-tubes in longitudinal section. × 600.



WIGGLESWORTH — LYCOPODIUM.

19

The Subaerial and Freshwater Algal Flora of the Tropics.

A Phytogeographical and Ecological Study.

BY

F. E. FRITSCH, D.Sc., PH.D., F.L.S.

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1. INTRODUCTORY.

EVER since Botany became an independent science the tropics have attracted numerous specialists, who have dealt with one or other aspect of the tropical flora. These regions of the world provide so many striking problems, that many of them, although subsequently recognized as evident enough in our parts of the world, were first noticed and studied in the tropics. The science of ecology in particular received its first stimulus from tropical observation, and until quite recent years we were better informed about the conditions of vegetation in many parts of the tropics than in our own country. At a fairly early period we find botanists devoting a little attention to the tropical algal flora, but the apparent similarity between the freshwater Algae of the tropics and those of our parts did not encourage extensive observation in view of the numerous more striking problems to be solved. It was only in the seventies and eighties of the last century that a marked tendency became evident to collect more extensive data regarding tropical Algae, and this has since then led to the publication of quite a number of algal floras from diverse parts of the tropics. The latter are mainly due to the labours of Nordstedt, Lagerheim, W. and G. S. West, Schmidle, Möbius, De Wildeman, and Lemmermann, although, as the bibliography at the end of this paper will show, a large number of botanists are responsible for occasional papers on this subject.

The general result of this work has been to show that there is a great degree of similarity between the algal flora of the tropics and that of other parts of the world. This is indeed what was to be expected in view of the fact that most of the Algae belong to the biological group of aquatics, and

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are, therefore, subject to much the same conditions in all parts of our globe [1]. But in spite of this uniformity of conditions, which is too obvious to need further comment, there are considerable minor diversities, which have been overlooked or not adequately considered, and no attempt has been made to correlate them with any peculiarity of the algal growth in different regions. Moreover, as regards the subaerial algal vegetation the influencing factors are markedly different in the tropics to those existing in our parts of the world, and here we have a much more fundamental and obvious difference in character. It is my object in the present paper to attempt an analysis of the existing data on tropical freshwater and subaerial Algae with the help of certain observations made in Ceylon. I hope to be able to show that the apparent similarity of algal growth all over the world is not quite as great as is generally imagined.

Very few of the existing tropical algal floras can be regarded as in any way representative of the region they deal with. Many of them are merely records of the occurrence of a few species in a certain district, and even the more extensive ones show marked signs of being incomplete in one direction or other. They are nearly all the result of casual collections made by botanical travellers occupied in the investigation of other problems, and the only exceptions are those of Welwitsch, Hansgirg, and De Wildeman. The two latter are, as far as I am aware, *the only* algologists who have had opportunities of collecting in person in the tropics, and even they were occupied with other investigations at the same time, and did not devote their whole attention to the algal flora. None of the existing floras aim at giving a true picture of the algal vegetation. They, of course, give us a record of a larger or smaller number of algal species found in a certain district, but they tell us nothing about the relative abundance in individuals of these species. The specific lists are of little use from this point of view, for, although a flora may include only a small specific element of a certain group, the number of individuals may be very great, and the group may play a very important part in the algal vegetation. Little blame attaches to the specialists who have worked out the collections, but one cannot suppress a certain amount of disappointment at the remissness of the collectors themselves in this respect. And this is not the only criticism that they call forth, for one misses in most of the floras adequate data as to locality and habitat, without which a flora becomes practically useless except from the geographical point of view. In very many cases the only information as to locality given is furnished by a mention of some province or town. Even some of the algologists do not appear to realize the necessity of as complete data as possible on these points (Turner, '92). I cannot in the very least agree with Schmidle ('00 A, p. 160) when he says: 'Es ist doch für die Wissenschaft völlig

[1] Cf. also Lemmermann, '06, p. 144 et seq.

gleichgültig, die genauen zufälligen Standorte solcher Ubiquisten zu kennen.' I think it very unlikely that for many of the forms enumerated by him without any mention as to locality that the latter is at all 'zufällig' (cf. also below). In view of these facts it is very difficult in most cases to obtain the kind of information we want from the tropical floras. I may take this opportunity of urging the importance of careful notes on relative abundance of species and habitat to all algal collectors, and more particularly to those who do not work out the results of their own collections, but pass them on to specialists for investigation. There is one other feature that it is well to pay attention to during the compilation of a flora, and that is the usual associates of certain forms to be considered in greater detail below. Data of this kind are to be found in a number of algal floras, but one completely misses them in others. And yet such data are hardly less important in aiding one to call up a picture of the algal vegetation of a given district than the relative abundance of the different species.

The subsequent remarks are based on the records contained in the literature enumerated at the end of this paper, and are supplemented and originally stimulated by observations made in Ceylon. The bibliography does not pretend to be absolutely complete, but I think that all the more extensive papers are included, and also all the smaller ones containing data bearing on the present subject. Only such papers are included as deal either entirely or partly with the flora of regions situated between the tropics of Cancer and Capricorn. This limitation is of necessity somewhat artificial, but I saw no other way of drawing a boundary.

In the course of the subsequent considerations it has frequently been necessary to enumerate all the records of a genus or group of genera. I have endeavoured to make these lists as complete and as accurate as possible in the hope that they may not only prove of service from the point of view of the present paper, but also as an epitome of our present knowledge of the distribution of these forms in the tropics. On the whole I have, however, refrained from dealing with these records critically, as such a treatment would have necessitated a much greater extension of this paper than seemed advisable.

2. The Systematic Composition of the Subaerial Algal Flora of the Tropics.

I have already elsewhere[1] pointed out the very different character of the subaerial algal vegetation in the tropics and in our parts of the world. The difference not only concerns the relative extent of development, but also the specific composition. With reference to the former point, my experience is gained from observation in the damp districts of Ceylon,

[1] Proc. Roy. Soc., Ser. B, vol. lxxix, 1907.

where the abundance of the subaerial algal covering is truly striking. I have no doubt that, wherever in other parts of the tropics the two necessary factors (temperature and moisture) combine, there exists a similar abundance of subaerial Algae. The literature indeed affords very few data to go upon. The few really extensive, and to some extent apparently complete, tropical algal floras do indeed enumerate a considerable number of subaerial Algae (see especially De Wildeman, '00; Gomont, '02; West and West, '93 and '97 A), but, as above mentioned, they give no data as to extent of occurrence. The only positive statement that I know of is as follows: Welwitsch [1] describes the prolific growth of a blue-green Alga, *Scytonema chorographicum*, Welw. (= *S. Myochrous* (Dillw.), Ag. var. *chorographicum*, W. and G. S. West), on the mountains of Pungo Andongo in Angola; this Alga causes a characteristic feature of the country, since it gives rise to a black colouration on these mountains (hence locally known as 'pedras negras' or black rocks) (cf. W. and G. S. West, '97 A, p. 303) [2]. Another '*Scytonema*' (= *Porphyrosiphon Notarisii*, Kütz.) plays an equally important part, according to Welwitsch, in the damp sands of the upper valley of the Cuanza River, where it 'frequently extends across the wide meadows, closely spread like a net over the soil, intergrown with the other herbs and smaller shrubs.' A perusal of Gomont's account of the Myxophyceae hormogoneae collected by the Danish expedition to the Gulf of Siam (Gomont, '01, p. 202 et seq.) also tends to show that there is a considerable subaerial algal element in these regions. The same impression is gained if we look over the records of Cyanophyceae in De Wildeman's algal flora of Buitenzorg (De Wildeman, '00). Other data in support of this view are given in the table on p. 244. As will be seen, there is little direct evidence to go upon, but it is sufficient to show that in all probability all damp tropical regions harbour a rich subaerial algal vegetation [3].

An examination of the abundant algal covering in the damp lowlands of Ceylon showed that it was practically entirely constituted by members of the Cyanophyceae (Myxophyceae). I have elsewhere [4] dealt with the many interesting biological peculiarities of this Cyanophyceous growth, and we are here only concerned with the systematic and phytogeographical sides of the question. There is practically no green element in the subaerial algal flora of the Ceylon lowlands (except *Trentepohlia*, see below, p. 242), the only marked exception observed being the growth on the subaerial portions of the stilt-roots of the mangroves at Kalutara (a form

[1] Welwitsch, Journal of Travel and Natural History, vol. i, 1868, pp. 22 et seq.

[2] Cf. also Warming, Ökologische Pflanzengeographie. German Edit., Berlin, 1896, p. 215.

[3] It is of course not impossible that under exceptional conditions even damp tropical regions may be poor in subaerial algal growth. Lemmermann ('05, p. 609) found very few aerophilous forms in his material from the Sandwich Islands, but it is open to question as to whether this is not due to imperfect collecting.

[4] Proc. Roy. Soc., Ser. B, vol. lxxix, 1907. Section *a* of this paper deals with the subaerial Algae (cf. also a forthcoming paper in the Geographical Journal, vol. xxix, 1907).

like *Pleurococcus*); but even here numerous Chroococcaceous colonies were intermingled, and threatened ultimately to completely crowd out the green forms. Occasional colonies of green forms in very subordinate amount are sometimes to be found amid the elements of the blue-green growth, but their number is so small that from a general point of view they are negligible. The great preponderance of blue-green forms is no doubt in part due to special favouring conditions (high temperature, humidity of the air), but in part also to the dominant factors in the tropics (intense light, frequency of and extreme degree of desiccation) being unsuitable to the growth of pure green forms. Light probably plays a great part in the exclusion of the latter, the successful blue-green element having a protective pigment in the form of the phycocyanin, which accompanies the chlorophyll in the cells.

We will now examine the literature with reference to this point. The statements quoted on p. 238 illustrate the fact that such records of an abundant occurrence of subaerial Algae in the tropics as exist refer to Cyanophyceae. All the more extensive algal floras show a relatively very large blue-green element, although no one actually mentions the dominance of the latter group in the subaerial vegetation [1]. An analysis of the tropical floras from this point of view will be found on p. 244, and it is unnecessary to say more on this subject here. The following is a list of the more important records of the occurrence of green subaerial forms in the tropics:—

1. *Pleurococcus crenulatus*, Hansg.—Schmidle, '97 B, p. 258 ('Am Holze der Regenrinnen, Savaii. Das getrocknete Zelllager ist **gelblichgrün**'); Lagerheim, '90, p. 6 (on branches of *Ilex scopulorum*).

2. *Pleurococcus Kützingii*, West.—West, '04, p. 287 ('Barbados.—Bay Estate; forming a **yellow-green** stratum about 1 mm. in thickness, with a species of *Lyngbya*').

3. *Pleurococcus miniatus* (Kütz.), Nägeli.—Lagerheim, '90, p. 6 ('En las piedras húmedas en el jardín botánico').

4. *Pleurococcus vulgaris*, Menegh.—West, '04, p. 287 ('Barbados.—Bay Estate'); De Wildeman, '97, p. 80 (Jard. bot. de Buitenzorg); West and West, '02 B, p. 200 ('On decomposing surface of gneiss, Matara'); Möbius, '95, p. 175 ('An einem Felsenabhang, Tubarão'); De Wildeman, '00, p. 109 ('Sur les écorces d'arbres, sur les murs, etc.—Jardin bot. de Buitenzorg'); Zeller, '73, p. 184 ('Rangoon, ad parietes hospitii "circuit house" dicti'); Hieronymus, '95, p. 22 (no habitat mentioned); Lagerheim, '90, p. 6 (tree-trunks); Bohlin, '97, p. 12 (Paraguay).

5. *Protococcus botryoides* (Kütz.), Kirchn.—West and West, '97 A, p. 239 ('Loanda. A light-green cover, often remaining dry, on the inner part of wooden water reservoirs').

[1] Lemmermann ('05, p. 609) comments on the absence of *Hormidium* and *Pleurococcus* from the subaerial algal flora of the Sandwich Islands.

6. *Protococcus caldariorum*, Magn.—Schmidle, '00 D, p. 8 (on leaves of trees, &c.; probably a stage in development of various Chroolepideae).

7. *Protococcus cinnamomeus*, Kütz.—Schmidle, '00 A, p. 161 (no locality given).

8. *Protococcus cohaerens*, Kütz.—Prain, '05, p. 329 (damp walls).

9. *Protococcus viridis*, Ag.—Schmidle, '00 A, p. 161 (no locality given); West and West, '97 A, p. 239 ('Loanda. Ad parietes domorum, &c., saepius humectatas in ipsa urbe Loanda, **ast sparsim.** Locus habitationis huius algae **austrum versus spectat**'); Dickie, '81, p. 125 ('Damp wood at Tabatinga, and at Serpa'); Schmidle, '97 E, p. 258 ('Auf faulendem Holz, dunkelgrün, Matautu, Savaii'); Lagerheim, '90, p. 4 (on trunks of *Prunus salicifolia*, H. B. K.).

10. *Stichococcus bacillaris*, Nägeli.—Lagerheim, '90, p. 5 ('En los muros húmedos en el jardín botánico y en otros lugares en Quito').

11. *Stichococcus flaccidus* (Kütz.), Gay.—West and West, '97 A, p. 239 ('Loanda. Inter *Protococcum botryoidem* (Kütz.), Kirchn.').

12. *Urococcus insignis* (Hass.), Kütz.—West and West, '97 A, p. 239 ('Huilla. In paludibus exsiccandis'); West and West, '93, p. 268 (on **mossy** trees, summit of Trois Pitons (4,500 ft.), Dominica).

13. *Cerasterias staurastroides*, W. and G. S. West.—West and West, '93, p. 268 (with *Scytonema javanicum*, Bornet, amongst **mosses** on lime-trees, Shanford Estate, Dominica).

14. *Gloeocystis gigas* (Kütz.), Lagerh.—West and West, '99, p. 285 ('On **trees in woods** round Roseau Lake, Dominica; with *Oocystis solitaria*, Wittr.).

15. *Gloeocystis rupestris* (Lyngb.), Rabh.—West and West, '97 A, p. 239 ('Pungo Andongo. Alga terrestris, ad ligna putrescentia in rupibus convallibus').

16. *Hormidium murale*, Kütz.—Schmidle, '00 A, p. 160 (no locality given).

See also *Rhizoclonium crassipellitum*, W. and G. S. West (p. 250), and *R. spongiosum*, Dickie (p. 251). Regarding *Trentepohlia*, see below, p. 242; terrestrial species of *Vaucheria*, p. 254.

This list shows that the records of green forms in the subaerial vegetation are meagre, especially when compared with the often very numerous records of subaerial blue-green forms (cf. table, p. 244). Moreover, such data as to habitat as are given point, at least in some cases, to some sort of protection against illumination; I have indicated such points by placing the words in black type, and do not think it necessary to offer further comment upon them at present.

The dominance of the Cyanophyceae in the subaerial algal vegetation of the tropics is thus manifest, and I cannot doubt that in other damp tropical parts they will be equally and perhaps even more strikingly developed than they are in Ceylon. A visit to any moist and hot greenhouse shows exactly the same thing, viz. great development of subaerial algal growth, which is almost purely blue-green. Everything points to most of the subaerial Cyanophyceae being more at home in a moist, hot atmosphere than in a cold one. Subsequent considerations will show that

the group of the blue-green Algae also plays a great part in the aquatic algal flora of the tropics (cf. p. 243). Amongst all the different groups of Algae probably none present so many unique features as do the Cyanophyceae. It is only necessary to recall the complicated cytology, the curious structure of the investment, the very characteristic propagation by means of motile filaments or hormogonia, and the peculiar heterocysts found in so many members of the group. Extensive study of the Cyanophyceae leads one to the gradual conviction that one is dealing with a very ancient phylum of the vegetable kingdom, which has in part retained a number of primitive features, although along other lines it has become highly specialized. There is little fossil evidence in support of this point of view, but so little is known about fossil Algae generally (except some of the calcareous forms) that the absence of such evidence cannot be regarded as in any way a proof against the ancient character of a group. A few doubtful fossils (e. g. *Zonatrichites*, Bornemann, and *Gloioconis Borneti*, Renault) have been referred to Cyanophyceae, and certain oolitic structures have been regarded as the result of the activity of members of this group, but these data are very fragmentary. Seward [1] discusses their possible presence in former periods as follows: 'Although our exact knowledge of fossil Cyanophyceae is extremely small, it is probable that such simple forms of plants existed in abundance during the past ages in the earth's history. Several writers have expressed the opinion that the blue-green Algae may be taken as the modern representatives of those earliest plants which first existed on an archaean land-surface. The living species possess the power of resisting unfavourable conditions in a marked degree, and are able to adapt themselves to very different surroundings. Their occurrence in hot springs proves them capable of living under conditions which are fatal to most plants, and suggests the possibility of their occurrence in the heated waters which probably constituted the medium in which vegetable life began [2].' In view of the exceeding abundance of this group of Algae in the tropics one can scarcely resist the assumption that their habitats in these regions are more in accord with their former conditions of existence than the habitats of temperate regions. A considerable number of Cyanophyceae have indeed become acclimatized to the changed conditions in these latter parts of the world, but the group is certainly prevalently tropical. Moreover, it was only in the damp tropics that many forms were able to leave the water and live a more or less terrestrial life; after all, the tropical subaerial habitats are semi-aquatic in the conditions they offer. The abundance of the blue-green group in the tropics is not the only evidence for the view that these regions of the world are more like their habitats in former epochs of the earth's history.

[1] Fossil Plants, vol. i, Cambridge, 1898, p. 131.

[2] Cf. also Warming, Ökologische Pflanzengeographie. German Edition, Berlin, 1896, p. 158.

In other respects also the Cyanophyceae show indications of being more favourably situated there. The heterocysts, in particular, point to such a conclusion, for in the greater portion of my material from Ceylon they show well-developed contents, often even coloured a deeper blue than the contents of the ordinary vegetative cells (e. g. in species of *Rivularia*[1]). The tropics no doubt afford a much more suitable field for the investigation of the blue-green group than do our parts of the world, and many of the problems connected with this group may find an adequate solution in the investigation of tropical material. It further does not seem at all unlikely that many of the Cyanophyceous forms of the tropics may pass through a more complete life-cycle than in the less favourable conditions of temperate regions. As an outcome of these considerations we may state that the Cyanophyceae are probably a group originally adapted to tropical conditions, and that there is some slight evidence for the view that they are the descendants of primitive algal forms, which flourished in earlier epochs of the earth's history under conditions somewhat similar to those obtaining in the damp tropics at the present day.

In talking of the minimal development of green forms in the subaerial algal flora of the tropics, the genus *Trentepohlia* was stated to be an exception. Species of this genus indeed play a great part in subaerial tropical vegetation[2], a statement based not only on my own observations in Ceylon, but also on the abundant records in the literature (*cf.* especially Karsten, '91; De Wildeman, '91, '94, '97, and '00; West and West, '02 B; Zeller, '73; Hariot, '89 and '92). The yellowish-red tufts or tresses of *Trentepohlia* are very abundant amidst the sombre Cyanophyceous growth in Ceylon, and form an agreeable interruption to its monotony. There can be little doubt that it is the screening action of the haematochrome in the cells of this genus that makes it alone successful amongst all the green forms, and this also points to intensity of illumination as being one of the most important factors excluding green forms from tropical subaerial habitats[3]. A very large number (about 40) of species of *Trentepohlia* have been described from the tropics, and in most cases the number of records is quite considerable (see also table on p. 244). Although so successful in the tropics, there seems no reason at present for looking upon this genus as essentially tropical; quite a large number of species are found in temperate regions, generally in well-illuminated habitats (especially abundant in alpine regions).

The abundant moisture in the damp tropics not only leads to the development of a very rich subaerial algal growth on tree-trunks, rocks,

[1] Cf. Fritsch, Studies on Cyanophyceae II. Beih. Bot. Centralbl., Bd. xviii, Abt. 1, 1905, p. 207, footnote 2.

[2] Lemmermann ('05, p. 609) states that *Trentepohlias* are wanting in the subaerial flora of the Sandwich Islands.

[3] Cf. also Fritsch, Proc. Roy. Soc., Ser. B, vol. lxxix, 1907.

walls, &c., but has also stimulated an epiphyllous tendency in many of the subaerial forms. Apart from epiphyllous Lichens and Liverworts, of the existence of which probably every one is familiar, we find a number of blue-green and green forms growing on the leaves of the higher tropical vegetation (cf. especially Schmidle, '97 C, '97 D, '97 F, and '98 B; Möbius, '88; Raciborski, '00). The blue-green forms are in great part the same as are met with in the general subaerial growth, and it is easy to comprehend how they invade moisture-covered leaves from neighbouring subaerial habitats. This phenomenon can be readily observed in the *Nepenthes*-house at Kew, where diverse blue-green forms settle down on the leaves of the plants cultivated there. The green forms, however, which have acquired the epiphyllous habit are on the whole much more specialized. They are all members of the Chroolepideae, the chief genera being *Cephaleuros*, *Mycoidea*, *Phycopeltis*, *Phyllactidium*, and *Phylloplax* (literature—Cunningham, '80; De Toni and Saccardo, '90; De Wildeman, '00; Karsten, '91; Möbius, '88; Raciborski, '00; Schmidle, '97 C, '98 B, '99; Ward, '84). Their structure is adapted to their mode of life, and like the species of *Trentepohlia*, their cells contain a yellow or red colouring matter, which exerts a screening action on the chlorophyll. The genera *Phyllosiphon* and *Phytophysa* (see especially De Wildeman, '00; Lagerheim, '92; and Raciborski, '00) have gone still further, since they penetrate the tissues of their substratum and lead a parasitic life. These diverse cases are of the utmost interest as showing the range of possibilities afforded by life in tropical subaerial habitats.

3. The Systematic Composition of the Submerged Aquatic Algal Flora of the Tropics.

A careful study of the freshwater algal flora of the tropics, such as I was able to undertake in Ceylon, brings out two points, viz.: (*a*) the dominance of the blue-green group, especially in the larger pieces of water, and (*b*) certain peculiarities of the green algal vegetation when considered as a whole. In dealing with the general characters presented by the submerged algal growth of the numerous (often large) freshwater tanks of Ceylon, I summarized my observations by stating[1] that 'whenever there is a well-developed algal flora in the considerable number of Ceylon tanks and lakes I have examined, there is *always a noticeable blue-green element which very frequently dominates the entire algal growth.*' The observations on which these conclusions are based are given in some detail in my paper on the general character of the freshwater algal flora of Ceylon (Fritsch, loc. cit.), and it will be sufficient to mention that of 46 tanks investigated 18 were absolutely dominated by Cyanophyceae with practically no green algal growth, while of the remainder 16 contained quite a considerable mass of

[1] loc. cit.

blue-green vegetation. As a general rule the smaller the dimensions of a tank the less pronounced is the blue-green element found to be. In other types of Ceylon freshwaters the dominance of the Cyanophyceae was equally pronounced (rock-pools and, to a less extent, the rice-fields) or not so marked (small pools and ditches, and especially wells). The prevalence of this group is no doubt, again, due to the high temperature of the water and the intensity of illumination. The importance of the former factor is recognized by a comparison between the lowlands and uplands of Ceylon, the latter having an aquatic flora in which the blue-green element is almost as casual as it is with us. The influence of the intense light is shown by the minor part played by the Cyanophyceae in the smaller waters, which are often more or less shaded by surrounding vegetation.

TABLE TO SHOW THE AVERAGE COMPOSITION OF TROPICAL FRESHWATER AND SUBAERIAL FLORAS.

Reference to Flora.	*Total No. of Species.*	*Total, less Desmids and Diatoms, &c.*	*No. of submerged green forms in reduced total.*	*No. of subaerial green forms in reduced total.*	*No. of submerged blue-green forms in reduced total.*	*No. of subaerial blue-green forms in reduced total.*	*Percentage of green forms in reduced total.*	*Percentage of blue-green forms in reduced total.*	*Percentage of blue-green forms in subaerial flora.*
De Wildeman ('00) . .	326	123 (1)	40	33[1] (T. 21)	27	23	59	41	41[1]
Dickie ('81)	101 (excl. Diatoms).	70 (3)	28	3 (T. 1)	18	21	44	56	88
Gomont ('02) . . . } West and West ('02 A) }	141	56	36	1 (T. 1)	6	13	66	34	93
Lemmermann ('06) . .	173	57	29		28		51	49	—
Möbius ('88)	32	23 (1)	14	1	4	4	65	35	80
Nordstedt ('78) . . .	113	69	39		30		57	43	—
Schmidle ('00 A) . . .	115	91 (1)	34		57		37	63	—
Schmidle ('02 B)[2] . .	57	47	10		37		21	79	—
Schmidle ('03 A) . . .	157	78	49		29		63	37	—
Turner ('92)	622	86	64		22		74	26	—
West ('04)	132	66	25	1	40		40	60	—
West and West ('93) .	63	43	4	4 (T. 2)	3	32	19	81	88
West and West ('97 A) .	298	159 (6)	76	8 (T. 2)	38	37	53	47	82
West and West ('02 B) .	394	99 (1)	58	8 (T. 7)	33	30	67	33	—
Zeller ('78)	126	120 (2)	45	12 (T. 9)	33	30	48	52	71

I am not aware of any direct statement in the literature as to the dominance of Cyanophyceae in the freshwaters of the tropics, although I feel convinced that Ceylon cannot be exceptional in this respect. Moreover a consideration of the few relatively complete floras that we possess distinctly points to this group being an important one in all parts of the

[1] The large number of subaerial green forms (even when the 21 *Trentepohlias* are excluded) is due to the enumeration of a considerable number of forms (belonging to Chroolepideae) found growing on leaves (cf. p. 243).

[2] A large number of the forms involved in this case are from hot springs.

tropics. The table on the preceding page is an analysis of the data in a number of these floras with reference to this point. The numbers, of course, only refer to species; there is no means of finding out the numbers of individuals. This is unsatisfactory, but it does show certain points. Since the smaller unicellular and colonial forms are as a rule quite subsidiary to the larger ones, I have thought it well, in order to get a better idea of the systematic composition of a flora, not to include the Desmids, Diatoms, Peridineae or Flagellates in these calculations.

In explanation of this table I have still to mention that the few freshwater species of Florideae have been included amongst the green submerged forms in order to avoid undue complication; where there are records of such forms the number of species is added in brackets after the reduced total (in the second column). Since the species of *Trentepohlia* are often a considerable increment in the green subaerial flora, their number has been indicated in brackets behind the actual total, in the fourth column (preceded by the letter T, which stands for *Trentepohlia*). In many cases the habitat-data were not sufficiently precise to enable one to distinguish between submerged and subaerial forms, so that the green and blue-green forms could only be included as a whole; in these cases it was, of course, impossible to give a percentage in the last column. All marine forms have been excluded. The percentages are calculated to the nearest whole number.

Before proceeding to discuss the features illustrated by this table, we will just take three European freshwater algal floras for comparison. For this purpose I have selected Messrs. W. & G. S. West's Freshwater Algae of the South of England[1]; Börgesen's Freshwater Algae of the Faeroes[2]; and Borge's Beiträge zur Algenflora von Schweden[3]. They illustrate the flora of regions which are sufficiently far apart and diverse in all except the temperate climatic conditions, and so there can be no fear of our dealing with a local aspect of European algal vegetation. Any other algal flora from our parts would give practically the same results as these do.

TABLE TO SHOW AVERAGE COMPOSITION OF FRESHWATER ALGAL GROWTH IN THREE EUROPEAN FLORAS.

Flora.	*Total No. of Species.*	*Total, less Desmids, Diatoms, &c.*	*No. of green forms in reduced total.*	*No. of blue-green forms in reduced total.*	*Percentage of green forms in reduced total.*	*Percentage of blue-green forms in reduced total.*
W. and G. S. West	588	255 (2)	194	61	76	24
Börgesen	323	148 (1)	107	41	72	28
Borge	472	191 (1)	149	42	78	22

[1] Journ. Roy. Microscop. Soc., 1897, p. 467 et seq.
[2] Botany of the Faeröes. Pt. I, Copenhagen, 1901, p. 198 et seq.
[3] Arkiv för Botanik, Bd. 6, No. 1, 1906.

It will be seen that the three floras from temperate regions show on the whole a considerably smaller percentage of blue-green forms than do the tropical floras; most of the exceptional cases among the latter (viz. Möbius, '88; Turner, '92; West and West, '02 B) are confessedly unrepresentative collections. Moreover, we must guard against attributing too much value to these figures, for they mean only relative abundance of species, and we know nothing about individuals. But the table of tropical floras does quite unmistakably seem to point to the Cyanophyceae being a very important constituent of the algal flora in all parts of the tropics (cf. also, for instance, the relative numbers of blue-green forms as compared with the total number of species in the two tables).

Not only is the blue-green element of great importance in the submerged macrophytic growth of tropical freshwaters, but it also plays a very important part in the Plankton. More than half of the large pieces of water in Ceylon, whose Plankton I examined, abounded in floating microscopic Cyanophyceae (especially *Clathrocystis*), which in many cases indeed were the sole components of the Phytoplankton. Reference to the literature, again, shows that Ceylon is in no way exceptional in this respect (cf. Schmidle, '03 A and '04 A; Lemmermann, '06, p. 147). I prefer, however, to defer further discussion on this subject, as I hope at an early date to deal with the Plankton contained in my collections from Ceylon.

The Cyanophyceae are a group of narrow forms, the diameter of the filamentous species rarely exceeding 15 μ, and very frequently being considerably less. That is a feature which may be of some advantage in water which is as poorly aerated as the ordinary tropical freshwaters are (cf. p. 252). A narrow filament will be better situated as far as gaseous exchange by diffusion is concerned than a broad one, and we may consequently expect that narrow forms will be more successful in the tropics than broad ones. As a matter of fact the entire freshwater algal flora of the tropics shows a preponderance of narrow forms. This is especially striking in the case of *Oedogonium* (cf. p. 265), a very important form in the green algal vegetation. The general prevalence of narrow forms is also due to absence or great scarcity of characteristic genera with broad filaments (*Vaucheria*, *Cladophora*). There are, however, two genera (*Spirogyra*, *Pithophora*) which furnish a considerable broad-celled contingent to the tropical aquatic algal flora, and of these *Spirogyra* is even highly abundant. This genus apparently gets on well in very poorly aerated water, whilst most of the freshwater algal genera have their broader representatives excluded from ordinary tropical freshwaters, or (probably) only developed under special conditions. This subject is discussed more fully in the following paragraphs. Confirmation for these theories must, of course, be sought in experiments, which will be undertaken in the near future.

Before concluding this section on the part played by the Cyanophyceae

in the tropics, I will give a list of the blue-green (subaerial and freshwater) genera as yet known to occur in these parts of the world. The number in brackets after each genus refers to the approximate number of species recorded; I should have liked to have given a full list of species and records, but space does not allow of that. Literature references are only given in the case of new genera. The genera are arranged according to Kirchner's classification (in Engler and Prantl, Die natürlichen Pflanzenfamilien, 1. Teil, Abt. 1 a, 1900, p. 45 et seq.):—

I. Chroococcaceae:—*Chroococcus*, Nägeli (17); *Synechococcus*, Nägeli (2); *Glococapsa*, Kütz. (15); *Placoma*, Schousboe (1); *Gloeothece*, Nägeli (5); *Aphanocapsa*, Nägeli (4); *Aphanothece*, Nägeli (9); *Microcystis*, Kütz. (6); *Clathrocystis*, Henfrey (2); *Gomphosphaeria*, Kütz. (1); *Coelosphaerium*, Nägeli (4); *Merismopedia*, Meyen (6); *Pilgeria*, Schmidle (n. gen. in Schmidle, '01 A, p. 53); *Tetrapedia*, Reinsch (10).

II. Chamaesiphonaceae:—*Xenococcus*, Thuret (1); *Dermocarpa*, Crouan (1); *Chamaesiphon*, A. Braun et Grunow (incl. *Sphaerogonium*, Rostaf.) (8).

III. Oscillatoriaceae:—*Oscillatoria*, Vauch. (28); *Arthrospira*, Stitzenberger (1); *Spirulina*, Turpin (10); *Phormidium*, Kütz. (22); *Proterendothrix*, W. and G. S. West (n. gen. in West and West, '97 A, p. 299); *Lyngbya*, C. A. Agardh (24); *Hypheothrix*, Kütz. (10); *Symploca*, Kütz. (8); *Porphyrosiphon*, Kütz. (1); *Hydrocoleum*, Kütz. (3); *Inactis*, Kütz. (2); *Schizothrix* (Kütz.), Gom. (16); *Polychlamydum*, W. and G. S. West (n. gen. in West and West, '97 A, p. 271); *Dasygloea*, Thwaites (1); *Microcoleus*, Desmazières (6).

IV. Nostocaceae:—*Nostoc*, Vauch. (20); *Nodularia*, Mertens (2); *Anabaena*, Bory (incl. *Sphaerozyga*, Ag.) (12); *Cylindrospermum*, Kütz. (6); *Aulosira*, Kirchner (1); *Microchaete*, Thuret (1); *Desmonema*, Berkeley and Thwaites (1).

V. Scytonemataceae:—*Plectonema*, Thuret (5); *Scytonema*, C. A. Agardh (40); *Tolypothrix*, Kütz. (14).

VI. Stigonemataceae:—*Mastigocladus*, Cohn (2); *Chondrogloea*, Schmidle (n. gen. in Schmidle, '02 B, p. 247) (2); *Hapalosiphon*, Nägeli (13); *Fischerella*, Gomont (1); *Stigonema*, C. A. Agardh (14); *Camptylonema*, Schmidle (n. gen. in Schmidle, '00 A, p. 181); *Capsosira*, Kütz. (1); *Nostochopsis*, Wood (1); *Myxoderma*, Schmidle (n. gen. in Schmidle, '02 B, p. 246) (1).

VII. Rivulariaceae:—*Leptochaete*, Borzi (1); *Calothrix*, C. A. Agardh (incl. *Lophopodium*, Kütz.) (18); *Dichothrix*, Zanardini (6); *Rivularia* (Roth), C. A. Agardh (incl. *Gloeotrichia*, J. Ag.) (9).

VIII. Camptotrichaceae:—*Camptothrix*, W. and G. S. West (n. gen. in West and West, '97 A, p. 269).

This list needs little further comment, since it shows the very large number of species and genera already recorded, and there is no doubt that their number will still be very considerably added to. Many of the species have been recorded from a large number of different localities, whilst in others the range at present appears to be restricted. Attention may also be particularly drawn to the great part played by Scytonema-

taceae and Stigonemataceae (especially *Scytonema*, *Tolypothrix*, *Hapalosiphon*, and *Stigonema*), and to the large number of genera of Oscillatoriaceae represented.

4. The Systematic Composition of the Green Algal Element in the Freshwaters of the Tropics.

There are certain marked peculiarities of the green algal element as found in tropical freshwaters, which are very manifest in Ceylon, and, judging by the data obtainable from existing floras, equally so in other parts of the tropics. Some of them are so striking that it is astonishing that there has been no previous comment upon them. Although the excess of Cyanophyceae is much the most prominent feature, the peculiar composition of the green algal flora supplements the particular stamp which the former already give tropical freshwater vegetation. The more important features presented by the green element are as follows :—

(*a*) The relative scarcity of *Cladophora* and *Rhizoclonium*, and the consequent scarcity of a number of characteristic epiphytes, which in our waters find one of their main substrata on these filamentous forms (e. g. *Cocconeis Placentula*, *Synedra splendens*).

(*b*) The replacement of these two genera by *Pithophora*.

(*c*) The great scarcity of *Vaucheria* (both aquatic and terrestrial species) and *Botrydium*.

(*d*) The relative scarcity of Ulotrichales and Confervales.

(*e*) The important part played by the genus *Spirogyra* combined with the relative scarcity of other Zygnemaceae, and the special systematic composition of the *Spirogyra*-element.

(*f*) The relative abundance of filamentous Desmids.

(*g*) The relative scarcity of broad species of *Oedogonium* (cf. above, p. 246).

These features have been dealt with briefly elsewhere [1], and a number of explanations to account for them are there suggested. It was, however, not possible to give full data in support of these suggestions, and I propose to do that here.

(i) The Freshwater Cladophoraceae of the Tropics.

Every algologist knows how commonly *Cladophora* or *Rhizoclonium* are to be met with in temperate freshwaters, and although no doubt in our parts conditions are often unfavourable for their development in certain waters, no considerable area can be surveyed without finding them well represented in the aquatic vegetation. In Ceylon matters are very different, and one can search for a long time before finding freshwater

[1] Fritsch, Proc. Roy. Soc., Ser. B, vol. lxxix, 1907.

representatives of these two genera. Although my collections are very extensive, and were made in many parts of the island, I met with very few occurrences of these Cladophoraceae. The most important habitats were two wells, which were examined; one near the streamlet at Matale, the other at Ambalangodda. The *Cladophora* in the former case was found in strongly **disturbed** (i. e. well-aerated) water, and was completely overgrown by Diatoms (mainly *Achnanthes*, also *Gomphonema* and *Synedra*) in a way which I cannot remember to have noticed anywhere else in the freshwaters of Ceylon. Besides these two wells the only other example of the occurrence of *Cladophora* that I met with was amongst the growth on the stony sides and bottom of the outflow of tank Nuwarawewa. At many points there was a covering of a close black growth forming finger-like patterns on the bottom, recalling the outline of a thallus of *Fucus*. This growth was a very short one, and difficult to detach from the rock. It consisted of a number of different forms, amongst which the *Cladophora* and a *Chantransia* with monospores were the most important. The *Cladophora* here was again growing in well-aerated water, and bore a rich growth of epiphytes. As to the other genus, *Rhizoclonium*, it was only found in the estuaries and lagoons. The observations thus made in Ceylon seemed to indicate that in the tropics both *Cladophora* and *Rhizoclonium* can only exist in well-aerated water (flowing or otherwise disturbed water). There are quite a considerable (though relatively to European waters small) number of records of these two genera to be found in tropical algal floras, and we shall do well to enumerate them before drawing further conclusions. They are all derived from the papers enumerated in the bibliography. The records are as follows:—

1. *Cladophora amplectens*, Welw. MS.—(*a*) West and West, '97 A, p. 36 (Loanda, on *Rhizophora*-roots, i. e. a **marine** form, at times submerged in well-aerated water).

2. *Cladophora* (*Spongomorpha*) *Beneckei*, Möb.—(*a*) Möbius, '93, p. 120 (Java, mostly from **rivers**, also apparently stagnant pools); (*b*) Schmidle, '00 A, p. 162 (bogs at Dadar near Bombay); (*c*) De Wildeman, '00, p. 83 (**flowing** and stagnant (?) water, Java).

3. *Cladophora crispata* (Roth), Kütz.—(*a*) West and West, '97 A, p. 36 (Loanda, from bottom of a **well**); (*b*) West, '04, p. 284 (Porter's Estate, Barbados); (*c*) Turner, '92, p. 163 (no locality mentioned!); (*d*) West and West, '96, p. 377 (Mwangadan **River**, S. of Fuladoga, Central Africa); (*e*) De Toni, '92, p. 272 (in **rushing stream**, Lava, Abyssinia); (*f*)? Möbius, '88, p. 240 (bathing establishment, Cabo; **river** and ditches of Coamo).

4. *Cladophora diluta*, v. Martens.—(*a*) Martens, '66, p. 20 (**rivers**, Philippine Islands).

5. *Cladophora dubia*, Schmidle.—(*a*) Schmidle, '97 E, p. 261 (forming incrustations on rocks overflown by **spring water**, Samoa; water possibly slightly brackish; see also Schmidle, '97 F, p. 285).

6. *Cladophora* (*Spongomorpha*) *fluviatilis*, Möbius.—(*a*) Möbius, '93, p. 119 (Java, from a **river**); (*b*) De Wildeman, '00, p. 84 (**flowing water**, Samarang, Java).

7. *Cladophora fracta* (Kütz.), Brand.—(*a*) Schmidle, '03 A, p. 86 (**littoral region** of Lake Nyassa, i.e., well-aerated water); (*b*) Schmidle, '00 A, p. 160 (no locality mentioned); (*c*) Möbius, '90, p. 1067 (no locality given); (*d*) Lemmermann, '05, p. 634 (Oahu, small grotto between Honolulu and Pali; probably **cold** water); (*e*) Lemmermann, '06, p. 160 (same habitat; also ditches between Honolulu and Wakiki).

8. *Cladophora glomerata*, Kütz.—(*a*) West and West, '02 B, p. 132 (Ceylon, in **running** water); (*b*) ? Möbius, '88, p. 240 (on rocks in **river** 'Morillos,' near Cayey); (*c*) Schmidle, '95, p. 294 (var. *genuina*, Rabh.; Indrapura, West Sumatra).

9. *Cladophora incurvata*, West and West.—(*a*) West and West, '02 B, p. 132 (Ceylon, from a pond in the Victoria Park, Colombo; water artificially aerated?).

10. *Cladophora javanica*, Kütz.—(*a*) De Wildeman, '00, p. 85 (in the **River** Brantas, Java).

11. *Cladophora* (*Spongomorpha*) *longiarticulata*, Nordst.—(*a*) Nordstedt, '78, p. 19 (in fish-ponds in valleys Nuanu and Paoa in island of Oahu); (*b*) Lemmermann, '05, p. 634 (sub *Clad. Nordstedtii*, De Toni; Oahu, marshes in Nuanu and Paoa).

12. *Cladophora luzoniensis*, v. Martens.—(*a*) Martens, '66, p. 20 (**rivers**, Philippines).

13. *Cladophora mollis*, Ag.—(*a*) Dickie, '81, p. 124 (near Manaos, &c.; from **river** water).

14. *Rhizoclonium africanum*, Kütz.—(*a*) Zeller, '76, p. 427 (ad **aquaeductum** montis Corcovado).

15. *Rhizoclonium biforme*, Kütz.—(*a*) Zanardini, '72, p. 152 (Sarawak-Marop, **in rivulis**).

16. *Rhizoclonium Berggrenianum*, Hauck.—(*a*) West and West, '93, p. 265 (var. *dominicense*, West and West; Dominica, in hot **stream** in crater of Grand'-Soufrière).

17. *Rhizoclonium crassipellitum*, West and West.—(*a*) West and West, '97 A, p. 35 (Loanda; a **terrestrial** species); (*b*) West, '04, p. 283 (var. *robustum*, G. S. West; Barbados, on **damp ground**).

18. *Rhizoclonium fontinale*, Kütz.—(*a*) Turner, '92, p. 163 (Northern India!); (*b*) Möbius, '88, p. 241 (isolated, on leaves of *Naias*, from **river** Quebra-Morillos, Cayey).

19. *Rhizoclonium hieroglyphicum*, Kütz.[1]—(*a*) West, '04, p. 283 (Trinidad, **in fountains**); (*b*) West, '04, p. 283 (var. *Kochianum*, Stockm.; Barbados, in **fresh**-water tank); (*c*) Borge, '99, p. 7 (Cordoba, Argentine!); (*d*) Schmidle, '03 A, p. 86

[1] According to Schmidle ('97 A), p. 3, *Conferva Sandvicensis*, Ag. belongs to *Rhizoclonium* and probably to *R. hieroglyphicum* (Kütz.), Stockm. var. *typicum* (De Toni), Stockm. With reference to its habitat Schmidle states (loc. cit.): 'Ich fand sie in dem von Lauterbach 1889 dort **gesammelten** Materiale häufig, teils in Wasser von verschiedenen Teichen, teils an **überrieselten Felswänden**.' The other records for *Conferva Sandvicensis* are West and West ('97 A), p. 34 (freq. in stagnis puris ad ripas flum. Bero, inter *Oedogonium* sp.); Nordstedt ('78), p. 18 (in Hawaii et in piscinis insulae Oahu); and Lemmermann, '05, p. 632 (Hawaii, Paoa and Nuanu; Oahu).

(Plankton of a **fountain-basin** near Langenberg); (*e*) Schmidle, '00 A, p. 160 (no locality mentioned); (*f*) Schmidle, '00 A, p. 160 (var. *macromeres* (Wittr.), Stockm.; no locality given); (*g*) Wille, '03, p. 5, sep. copy (isolated filaments; hot **springs**, Nhaondue; pools near Mutadzi); (*h*) Schmidle, '97 E, p. 260 (brackish water, Savaii); (*i*) Borge, '06, p. 4 (at height of 4,000 metres, i.e. **cool water**; Laguna, Colorado); (*j*) Heydrich, '94, p. 271 (var. *Julianum* (Menegh.), Rabenh.; rocks, S. Formosa); (*k*) Schmidle, '95, p. 296 (var. *striatum*, Schmidle; probably **flowing water**, West Sumatra); (*l*) West and West, '99, p. 281 (var. *tortuosum*, Stockm.; in small **stream**, Roseau Valley, Dominica); (*m*) ? Grunow, '67, p. 37 (freshwater, Rio de Janeiro; also var. *crassior* from Tahiti).

20. *Rhizoclonium spongiosum*, Dickie.—(*a*) Dickie, '81, p. 124 (forming spongy masses ¼ to ½ inch thick on wet sandstone rocks near Manaos, i.e. a **terrestrial** form).

N.B.—This list does not absolutely exhaust all the records of freshwater species of these two genera found in the works enumerated in the bibliography at the end of this paper. All those in which a definite habitat was given are included, but a certain number of others, which were extremely vague, have been left out. A number of the records of *Cladophora*, published prior to Wittrock's monograph of the Pithophoraceae (in Nova Acta Reg. Soc. Sc. Upsala., Ser. 3, 1876), really refer to species of *Pithophora* (cf. loc. cit., pp. 2 and 3)—possibly even some of the later ones, although *Pithophora* is on the whole quite easily distinguished from *Cladophora*, even in the sterile condition, by its habit and its mode of branching (loc. cit., p. 4).

Where details as to habitat are given, the important feature from the present point of view is indicated by the use of black type.

I think it is scarcely necessary to make much further comment on this list. It shows that in a large number of cases the individuals of these two genera were found in flowing water or in water which was better aerated than the ordinary freshwaters of the tropics are. The remaining cases cannot be taken as a decisive proof to the contrary until such habitats have been fully studied. Moreover, as in the majority of cases there is absolutely no mention of the amount of the Alga, we do not know whether we are dealing with an abundant form or merely with an isolated occurrence. A number of the localities, which do not obviously point to well-aerated water, are very suggestive of artificial aeration (e. g. that inhabited by Messrs. West and West's *Cladophora incurvata*[1]).

It will have been gathered from the preceding remarks that I am attempting to show that *Cladophora* and *Rhizoclonium* are relatively so scarce in tropical freshwaters, because the ordinary waters are too poorly aerated to admit of their existence. Owing to their higher temperature tropical waters contain a far smaller amount of the essential gases (oxygen and carbon dioxide) than do those of temperate regions. In

[1] I unfortunately omitted to visit this locality during my stay in Ceylon.

illustration of this statement we may take the following data from Forel[1] dealing with the relative amounts of dissolved atmospheric gases (per litre) as found in waters (Lake of Geneva) of different temperatures:—

Temperature.	*O.*	*N.*	CO_2.
5° C.	7·3 cc.	13·6 cc.	0·6 cc.
20° C.	5·7 cc.	10·7 cc.	0·3 cc.

The lowest temperature attained by the lowland waters of Ceylon I found to be 25° C., so that the amount of dissolved oxygen will probably be less than 5 cc. Both *Cladophora* and *Rhizoclonium* have thick coarse walls, which are not suitable for a ready diffusion of gases from the surrounding water, and even in temperate regions these two genera seem to flourish best in waters which are not absolutely stagnant. Moreover, their period of maximum abundance generally falls into the cold part of the year, and although not completely absent in the summer they are much reduced in quantity. From this point of view, therefore, we need not be surprised to find the two genera in question very scantily represented in tropical freshwaters, and practically confined to such as are in some way or other well aerated. It is of course not impossible that certain species of *Cladophora* or *Rhizoclonium* can occasionally frequent stagnant water in the tropics, but up to the present there is no conclusive evidence on this point.

I found the order Cladophoraceae in Ceylon mainly represented by species of *Pithophora*. They cannot be said to be really common forms, although frequent enough to be a sensible constituent of the algal flora. As regards their habitat, they appear to favour mainly the smaller pieces of water, and were not met with in the larger tanks. Reference to the literature shows a remarkably small number of records of species of this genus[2], which does not at all tally with my observations in Ceylon. Can it be that some of the records of *Cladophora* above enumerated really concern species of *Pithophora*? I do not think it very likely, but it is well to keep the possibility in view. It does not, however, seem at all probable that Ceylon is exceptional in the relative abundance of *Pithophora* as compared with the other Cladophoraceae, and we must await a careful investigation of the algal flora in some other part of the tropics before this point can be absolutely settled. In its relatively thin walls *Pithophora* certainly seems better suited than the other Cladophoraceae for life in tropical waters, and in Ceylon I frequently observed it in water which

[1] Allgemeine Biologie eines Süsswassersees, in Zacharias, Die Tier- und Pflanzenwelt des Süsswassers. Leipzig, 1891, p. 15.

[2] The more important species are: *Pithophora aequalis*, Wittr.; *P. affinis*, Nordst.; *P. clavifera*, Schmidle; *P. Cleveana*, Wittr.; *P. oedogonia* (Mont.), Wittr.; *P. pachyderma*, Schmidle; *P. polymorpha*, Wittr.; *P. radians*, W. and G. S. West; *P. Reineckei*, Schmidle; *P. Roettleri* (Roth.), Wittr.; *P. sumatrana* (Mart.), Wittr.; *P. variabile*, Schmidle.

was absolutely stagnant[1]. In addition to its thinner walls and often narrower filaments, however, *Pithophora* differs in another respect from the other members of the Order. It is capable of forming specialized resting-spores (akinetes), which may be a feature of great advantage to it in view of the frequency of rather sudden desiccation in tropical waters, especially in the smaller ones, in which *Pithophora* was almost alone observed in Ceylon. It is just possible that this may be a second factor contributing towards the scarcity of *Cladophora* and *Rhizoclonium*, and that their means of existence during times of exposure and desiccation may not be suited to the exigencies of the tropics. This factor is, however, certainly in no way as important as the aeration of the water.

Pithophora is certainly a prevalently tropical genus—in fact, all the European records are subject to the suspicion of the forms found there being introduced[2].

With the great scarcity of *Cladophora* and *Rhizoclonium* in tropical freshwaters goes hand in hand the lack of one of the most important substrata for aquatic algal epiphytes. Every one is probably familiar with the dense covering of characteristic Diatoms (*Cocconeis*, *Gomphonema*, *Synedra splendens*, &c.) found in temperate regions on the filaments of the two genera in question, often completely enshrouding them in an heterogeneous brown mass. Scarcely any other filamentous Alga (saving perhaps *Vaucheria*, which is also very scarce in tropical waters, cf. below) approaches the Cladophoraceae in this respect, and in tropical waters, where these genera are not represented, epiphytic algal growth is rarely at all well developed. There are, however, other reasons to account for this. A large number of the Diatoms generally seem to prefer cold to warm water, and with us mostly attain their maximum in the cold winter months, and steadily decrease in amount as the water becomes warmer in spring. There are, however, also a number of Diatoms which exist in great numbers in much warmer waters, as is shown by the numerous records of their occurrence in hot springs. These latter, besides including cosmopolitan species, consist mainly of a few probably specialized forms (filamentous Diatoms seem to be predominant). In waters of intermediate temperature, such as are afforded by those of the tropics, the conditions seem unfavourable both for the colder forms and for the hot-spring forms, and the Diatom-flora is generally poorly developed. In Ceylon I only met with Diatoms in striking abundance in river-waters, as epiphytes on Cladophoraceae in wells, and in the hot springs (Kannia near Trincomalee).

[1] That *Pithophora*, however, also has a taste for aerated water is shown by the fact that it was found commonly in the water of wells examined in Ceylon.

[2] There are no data as yet forthcoming as to the distribution of Schmidle's new genus *Chaetonella* with *C. Goetzei* (Schmidle, '02 B, p. 253). Further observation may show it not sufficiently distinct to warrant generic separation. The habitat is in accord with the general remarks made above ('Pfütze in der **Nähe der Brandung**').

In minor numbers they are generally present in every collection, although certain types of waters are practically destitute of them.

From what I have seen in Ceylon it seems as though the epiphytic Diatoms of tropical waters were not in general quite the same as those found in our parts of the world. The species of *Cocconeis* in particular are very rare, whilst *Achnanthes* seems to play a very much more important part than with us. Most of the tropical floras, however, give us little or no information about Diatoms[1], and I prefer, therefore, to omit further speculation on this subject until more data are available for general verification. In the case of abundant occurrence of tropical freshwater Diatoms it is also of course of the utmost importance to have full descriptions of the habitats. It seems as though the small amount of dissolved gases in the water may again be the governing factor in determining the scarcity of this group in stagnant tropical waters.

(ii) The Freshwater and Terrestrial Siphoneae of the Tropics.

The genus *Vaucheria* is quite evidently very poorly represented in the tropics. In the case of Ceylon I have not as yet found a single example of its occurrence in the lowlands, although (in the course of a six days' stay only) in the uplands, at a height of 6,000 feet and more, both aquatic and terrestrial forms were met with. The literature is equally poor in records of this genus; the following are extracted from the papers in the bibliography:—

1. *Vaucheria geminata* (Vauch.), DC.—(*a*) Hieronymus, '95, p. 23 (in ditches, &c.).

2. *Vaucheria humicola*, Lagerh.—(*a*) Lagerheim, '90, p. 14 (in terra humida horti botanici Quitensis[2]).

3. *Vaucheria repens* (Hass.), Klebs.—(*a*) Schmidle, '02 A, p. 64 (forma *nasula*, Schmidle; on damp stones in streamlet Njasoso and on damp soil on the banks; Africa).

4. *Vaucheria sessilis* (Vauch.), DC.—(*a*) West and West, '97 A, p. 235 (Golungo Alto[2], Africa. In fossis exsiccatis adhuc humidis in Varzea pone dom. Isidni; covers in Aug. and Sept. all the half-dried-up ditches of the plain); (*b*) West and West, '97 A, p. 235 (var. *monogyna*, W. and G. S. West; Golungo Alto[2], Africa. Ad margines stagnorum rivi Cuango, Aug. 1857); (*c*) Zeller, '73, p. 186 (var. *cespitosa* Ag.; Pegu, Yoma centralis, Khayeng-mathay-choung, Burma. Var. *repens*, Hass., ibidem, in limo siccescente); (*d*) Zeller, '76, p. 427 (var. *subarticulata*, Zeller; no locality).

Sterile species of *Vaucheria* are recorded by (*a*) Dickie, '81, p. 125 (on moist earth, Porto Salvo, Rio Purus); (*b*) Schmidle, '03 A, p. 86 (Plankton of Nyassa, near Langenburg!); (*c*) De Toni, '92, p. 272 (in rivulis, inter Gheleb et Maigerghebit); (*d*) Turner, '92, p. 155 (in fragmentary condition only; Bengal, Central India!);

[1] Cf., however, De Wildeman ('00), West and West ('02 B), Lemmermann ('05 and '06), Gutwinski and Chmielewski ('06), Dickie ('81).

[2] These localities are situated at a high altitude.

(*e*) West, '04, p. 284 (Roy. Bot. Gards., St. Anns, Trinidad!); (*f*) Hieronymus, '95, p. 23 (in a drain and small waterfall); (*g*) Martelli, '86, p. 151 (Sciotel, Alle falde della Zedamba).

These are all the records there are, and while they serve to show that *Vaucheria* does occur in the tropics, they point to conditions being on the whole unfavourable to it. An examination of the records just enumerated also shows that in the majority of cases the *Vaucheria* found was a terrestrial form (often growing at a higher altitude), and not aquatic. This seems to indicate that the water is particularly unsuitable for these forms in the tropics, and, bearing in mind the broad filaments generally found in this Alga, one inclines to the assumption that the same factor is responsible for the great scarcity of aquatic *Vaucheria*, as was suggested as the probable cause for the rarity of tropical freshwater *Cladophoras* and *Rhizocloniums*, viz. the small amount of dissolved oxygen in the water. This view receives some support from the fact that marine species of *Vaucheria* (i. e. forms growing in well-aerated water) are apparently not at all uncommon in the tropics (cf., for instance, De Wildeman, '00, p. 89). There are, however, other special features in the genus *Vaucheria*, which may be an unsuitable equipment for tropical life. The assimilatory process is decidedly different to that of the majority of green Algae,[1] the reserve-product being oil and the chloroplasts being yellowish and devoid of pyrenoids. It is possible that in some way or other assimilation of this type may not go on well amid tropical conditions (cf. below)—perhaps it is more successful on the land than in the water.

Although Ceylon affords many likely localities for *Botrydium* I did not find it, and other collectors have been almost equally unsuccessful. There are only three records, viz.:—*B. argillaceum*, Wallroth (Martens, '70, p. 298; 'in praeruptis viarum argillosis, in fossis limosis ad Ypanema, Prov. S. Pauli, tempore pluvio'); *B. granulatum* (L.), Grev. (Lagerheim, '90, p. 7; 'En los muros de las calles exteriores de Quito'); and *B. granulatum* (L.), Grev. var. *aequinoctiale*, W. and G. S. West (West and West, '97 A, p. 235; 'Loanda. Non infrequens in territor. Loandensis terris humidis argillaceo-arenosis, latas plagas imo urbis ipsius plateas, etc., obtegens; mox post pluvias copiosas nascens, citoque tempore sicco disparens'). *Botrydium* is, of course, a form which is fairly readily overlooked, and my main object is to draw attention to the few records. As far as the assimilatory process is concerned *Botrydium* is, however, in the same position as *Vaucheria*.

(iii) The Confervales in the Tropics.

In dealing with *Vaucheria* and *Botrydium* one naturally also turns one's attention to the Confervales, a group to which the latter genus is now generally referred, whilst the inclusion of *Vaucheria* in the Confervales is

[1] Cf. Oltmanns, Morphologie und Biologie der Algen. Zweiter Band. Jena, 1905, p. 147.

also advocated by some. In looking over the records of tropical freshwater Confervales one comes to the conclusion that this group is also one which plays no great part in the tropics, though certainly represented by a number of forms. The records are as follows:—

1. *Characiopsis ellipsoidea*, G. S. West.—(*a*) West, '04, p. 288 (near Bridgetown, Barbados; epiphytic on *Chara*, sp.).

2. *Conferva Ansonii*, Agardh.—(*a*) Schmidle, '00 D, p. 10 (Marianne Islands, **in a spring**).

3. *Conferva bombycina*, Ag.—(*a*) West and West, '93, p. 265 (f. *minor*, Wille; in cold, warm, and hot **streams**, crater of Grand'-Soufrière, Dominica); (*b*) West and West, '97 A, p. 34 (f. *minor*, Wille; Pungo Andongo; ad ramulos Podostemacearum in **rivulis**, Casalalé); (*c*) De Toni, '92, p. 271 (in alveo **flumin.** Anseba, pr. Arbasciko); (*d*) Möbius, '95, p. 175 (in a small lake, **2,300 metres altitude**, Itajahy); (*e*) Lemmermann, '05, p. 631 (var. *minor*, Wille; Oahu, Maluhia!); (*f*) Prain, '05, p. 329 (**River** Hughli, near Kidderpore; Botanic Garden, Shibpur, in ponds); (*g*) Zanardini, '72, p. 151 (Sarawak, **in rivulis** montis Mattang); (*h*) Lagerheim, '90, p. 3 (wet soil in botanic gardens, Quito).

4. *Conferva fontinalis*, Berk.—(*a*) Turner, '92, p. 162 (Northern India!); (*b*) Zanardini, '72, p. 151 (var. *ochracea*; Sarawak–Marop, **in rivulis**).

5. *Conferva funicularis*, Agardh.—(*a*) Schmidle, '00 D, p. 10 (Mariana Islands, **in the river** Agana).

6. *Conferva Glaziovii*, Zeller.—(*a*) Zeller, '76, p. 426 (in *Vaucheria sessili* parasitica).

7. *Conferva sandvicensis*, Ag.—See footnote on p. 250.

8. *Conferva tenerrima*, Kütz.—(*a*) Turner, '92, p. 163 (Northern India!); (*b*) Schmidle, '02 B, p. 252 (Masote, in **river** Siwa, but in stagnant water).

9. *Ophiocytium Arbuscula*, A. Br.—(*a*) Lagerheim, '90, p. 13; (*b*) Bohlin, '97, p. 30 (Paraguay!).

10. *Ophiocytium biapiculatum*, Hieron.—(*a*) Schmidle, '03 A, p. 83 (quiet bay of Mbasi-river near its point of inflow into Lake Nyassa); (*b*) Hieronymus, '95, p. 22 (marshes).

11. *Ophiocytium bicuspidatum*, Lemm.—(*a*) West and West, '02 A, p. 160 (in muddy ricefield).

12. *Ophiocytium capitatum*, Wolle.—(*a*) Lagerheim, '93, p. 159 (with *Utricularia inflexa*, Forsk.); (*b*) West and West, '02 B, p. 130 (var. *longispinum*, Lemmermann; running water, Royal Botanic Gardens, Peradeniya).

13. *Ophiocytium cochleare*, Näg.—(*a*) West and West, '95, p. 82 (Madagascar); (*b*) Borge, '99, p. 9 (Argentina, Cordoba!); (*c*) Turner, '92, p. 154 (no locality); (*d*) De Wildeman, '97, p. 79 (Dutch Indies); (*e*) Wille, '84, p. 11 (Caldas!); (*f*) Schmidle, '03 A, p. 83 (ditch near Langenburg); (*g*) Schmidle, '95, p. 296 (no locality); (*h*) De Wildeman, '00, p. 106 (ditches, marshes); (*i*) Nordstedt, '80, p. 12 (in *Utricularia Eckloni*); (*j*) Gutwinski, '02 A, p. 577 (var. *bicuspidatum*, Borge); (*k*) Hieronymus, '95, p. 22 (marshes); (*l*) Lagerheim, '90, p. 13 (en los pantanos en S. Rita); (*m*) Bohlin, '97, p. 29 (Paraguay, Matto Grosso; also vars. *bicuspidatum* and *longispinum*).

14. *Ophiocytium cuspidatum* (Bail.), Rabh.—(*a*) Borge, '99, p. 9 (Guiana !).

15. *Ophiocytium gracilipes*, A. Br.—(*a*) Lemmermann, '05, p. 631 (Oahu, small grotto between Honolulu and Pali); (*b*) Gutwinski, '02 A, p. 577; (*c*) Lemmermann, '06, p. 160 (same as *a*); (*d*) Bohlin, '97, p. 30 (Matto Grosso !).

16. *Ophiocytium majus*, Näg.—(*a*) Turner, '92, p. 154 (Northern India !); (*b*) West and West, '97 A, p. 236 (Pungo Andongo; in stagnis prope Anbilla, Condo); (*c*) Lagerheim, '93, p. 159 (also var. *gordianum*, Istv.; with *Utricularia inflexa*, Forsk.); (*d*) Borge, '96, p. 7 (Johnstone River, Queensland).

17. *Ophiocytium parvulum*, A. Br.—(*a*) West and West, '02 A, p. 161 (in muddy rice-field); (*b*) West and West, '97 A, p. 236 (Pungo Andongo ; in stagnis prope Anbilla, Condo); (*c*) West and West, '02 B, p. 131 (stream, Victoria Park, Colombo); (*d*) Schmidle, '95, p. 296 (no locality); (*e*) Schmidle, '02 A, p. 64 (Abo River); (*f*) Lagerheim, '90, p. 13 (en los pantanos en S. Rita); (*g*) Bohlin, '97, p. 29 (Paraguay, Matto Grosso !).

The most important form, as far as number of species and records go, is thus *Ophiocytium*. *Conferva* (especially if we follow Schmidle and exclude *C. sandvicensis*, Ag.) is not very well represented, and a perusal of the records of this genus shows that, like *Rhizoclonium* and *Cladophora*, it favours well-aerated water (rivers and waters at a high altitude). In Ceylon I have only met with occasional filaments of *Conferva*, although future more thorough investigation is sure to disclose a number of the unicellular forms; certainly, however, none of them is abundant. One cannot help thinking of the peculiar type of assimilation as another explanation of the scarcity of *Conferva* (i. e. in addition to its apparent desire for well-aerated water). As in the case of *Vaucheria* and *Botrydium*, however, this point must remain open until we have far more precise data as to the kind of tropical habitats frequented by these forms.

(iv) The Ulotrichales in the Tropics.

It is not quite apparent from existing data whether the Ulotrichales are really rarer in the tropics than in temperate waters. I am tempted to such a conclusion by my observations in Ceylon, where they certainly, as far as the lowland waters are concerned, are relatively scarce. Messrs. W. and G. S. West, in their work on the freshwater Algae of Ceylon (West and West, '02 B, p. 124), also comment on this fact; they say: 'Very few Algae were observed belonging to the families Confervaceae and Ulotrichaceae; this we are unable to account for, as many of the collections were from suitable localities for these plants.' It again does not seem likely that Ceylon is exceptional, although possibly poorer in these forms than other parts of the tropics. As far as existing data go, there is nothing in the records of tropical Ulotrichales to point to their being bound to special conditions, and I think, therefore, that it will be sufficient to enumerate the species of this group so far recorded from tropical freshwaters. The

different genera are arranged according to Blackman and Tansley's 'Revision'[1]:—

I. *Ulotrichaceae*:—

1. *Ulothrix minutula*, Kütz. (Nordstedt, '78, p. 22; Lemmermann, '05, p. 632); *U. oscillarina*, Kütz. (Wille, '84, p. 25; ? Dickie, '81, p. 124); *U. pectinalis*, Kütz. (Prain, '05, p. 329); *U. subtilis*, Kütz. (Schmidle, '03 A, p. 85; Zeller, '73, p. 189; Lemmermann, '05, p. 632); *U. subtilis*, Kütz., var. *variabilis* (Kütz.), Kirchn. (West, '04, p. 282; West and West, '96, p. 377); *U. tenuis*, Kütz. (Möbius, '95, p. 174); *U. tenerrima*, Kütz. (Turner, '92, p. 162); *U. zonata*, Kütz. (Turner, '92, p. 162).

1 *a*. *Hormiscia*[2] *aequalis* (Kütz.), Rabenh. (Schmidle, '02 A, p. 62; Schmidle, '00 D, p. 9); *H. oscillarina* (Kütz.), De Toni (De Toni, '92, p. 271; Schmidle, '02 A, p. 62); *H. rigidula*, Kütz. (Schmidle, '00 A, p. 160); *H. subtilis* (Kütz.), De Toni (West and West, '97 A, p. 33; Nordstedt, '97, p. 131); *H. subtilis*, var. *stagnorum*, Kirchn. (Schmidle, '00 A, p. 160); *H. subtilis*, var. *tenerrima*, Kirchn. (West and West, '02 B, p. 129; Schmidle, '97 E, p. 258); *H. subtilis*, var. *thermarum* (Wartm.), Rabenh. (Schmidle, '02 A, p. 62); *H. subtilis*, var. *variabilis*, Kirchn. (Schmidle, '00 A, p. 160; West and West, '97 A, p. 33); *H. zonata*, Aresch. (Möbius, '93, p. 118; De Wildeman, '00, p. 59).

2. *Hormidium*, see p. 240.

3. *Uronema confervicolum*, Lagerh. (West, '04, p. 283); *U. confervicolum*, Lag. var. *javanicum*, Möb. (Möbius, '93, p. 118; De Wildeman, '00, p. 58).

II. *Prasiolaceae*:—

4. *Schizogonium tenuissimum*, Zeller (Zeller, '73, p. 189).

III. *Microsporaceae*:—

5. *Microspora abbreviata* (Rabh.), Lag. (Schmidle, '03 A, p. 85; West and West, '02 A, p. 160; West and West, '97 A, p. 34; Schmidle, '02 A, p. 62; West and West, '99, p. 280); *M. amoena* (Kütz.), Rabenh. (West and West, '97 A, p. 34; Schmidle, '02 A, p. 62); *M. De Toniana*, Lagerh. (Lagerheim, '93, p. 157); *M. floccosa*, Thur. (? Möbius, '93, p. 119; West and West, '02 B, p. 130; De Wildeman, '00, p. 63); *M. fontinalis* (Berk.), De Toni (West and West, '97 A, p. 34); *M. Löfgrenii*, Nordst. (West and West, '97 A, p. 34); *M. pachyderma* (Wille), Lagerh. (West and West, '99, p. 280; Borge, '96, p. 6); *M. stagnorum* (Kütz.), Lagerh. (Lagerheim, '90, p. 12); *M. Willeana*, Lagerh., var. *abyssinica*, De Toni (De Toni, '92, p. 271); *M. Wittrockii* (Wille), Lagerh. (West and West, '97 A, p. 34; Lagerheim, '90, p. 12).

V. *Chaetophoraceae*:—

6. *Stigeoclonium amoenum*, Kütz. (Nordstedt, '97, p. 132); *S. falklandicum*, Kütz. (Nordstedt, '78, p. 22; Lemmermann, '05, p. 632); *S. macrocladium* (Nordst.), Schmidle, var. *tomentosum*, Schmidle (Schmidle, '00 A, p 162); *S. plumosum*, Kütz. (Dickie, '81, p. 123); *S. protensum*, Kütz. (? Turner, '92, p. 163);

[1] New Phytologist, vol. i, 1902.

[2] I have thought it better to place the records under the two genera *Ulothrix* and *Hormiscia* as they are found in the literature, although a number of the species are of course synonymous.

S. Rangoonicum, Zeller (Zeller, '73, p. 191); *S. spicatum*, Schmidle (De Wildeman, '97, p. 73; De Wildeman, '00, p. 61; Schmidle, '95, p. 294); *S. tenue*, Rabh. (Schmidle, '00 A, p. 160; Schmidle, '01 A, p. 46; Zeller, '73, p. 191; Möbius, '92, p. 24); *S. thermale*, A. Br. (Schmidle, '01 A, p. 46); *S. uniforme* (Ag.), Rabenh. (Hieronymus, '95, p. 23).

7. *Chaetophora elegans* (Roth), Ag. (Lagerheim, '90, p. 11); *C. pisiformis*, Ag. (Zeller, '73, p. 191); *C. radians*, Kütz. (Zeller, '73, p. 191); *C. stricta*, Zeller (Zeller, '73, p. 191); *C. tuberculosa* (Roth), Hook. (De Wildeman, '97, p. 73; De Wildeman, '00, p. 61; Zeller, '73, p. 191).

8. *Draparnaldia glomerata* (Vauch.), Ag. (Lagerheim, '90, p. 11); *D. macroclada*, Nordst. (Nordstedt, '78, p. 22; Lemmermann, '05, p. 632).

9. *Aphanochaete Braunii* (Näg.) (De Wildeman, '97, p. 72; De Wildeman, '00, p. 59); *A. crassisetum* (W. and G. S. West) (West and West, '02 B, p. 130); *A. pilosissima*, Schmidle (West and West, '02 B, p. 125); *A. polychaete* (Hansg.), Fritsch (Lagerheim, '93, p. 157); *A. repens*, A. Br. (West and West, '02 A, p. 158; Nordstedt, '78, p. 23; Borge, '96, p. 6; Lemmermann, '05, p. 632; Lemmermann, '06, p. 160; Lagerheim, '90, pp. 3 and 11).

10. *Endoderma immane*, Schmidle (Schmidle, '00 A, p. 167; Schmidle, '00 B, p. 17); *E. Pithophorae*, G. S. West (West, '04, p. 283); *E. polymorpha*, G. S. West (West, '04, p. 283); *E. Reineckei*, Schmidle (Schmidle, '97 E, p. 259; West and West, '02 B, p. 129).

11. *Psephotaxus lamellosus*, W. and G. S. West (West and West, '97 A, p. 33).

12. *Chaetopeltis minor*, Möb. (Schmidle, '01 A, p. 46).

VII. *Chaetosphaeridiaceae*:—

13. *Chaetosphaeridium globosum* (Nordst.), Klebahn (Nordstedt, '78, p. 23; Wille, '84, p. 25; Lemmermann, '05, p. 633); *C. Pringsheimii*, Klebahn (Borge, '99, p. 7; De Wildeman, '97, p. 73; De Wildeman, '00, p. 60; Schmidle, '01 A, p. 46).

VIII. *Coleochaetaceae*:—

14. *Coleochaete irregularis*, Pringsh. (West and West, '95, p. 42; Schmidle, '01 A, p. 46; Borge, '96, p. 3; Nordstedt, '78, p. 23; Lemmermann, '05, p. 633); *C. javanica*, De Wildeman (De Wildeman, '97, p. 52; De Wildeman, '00, p. 48); *C. orbicularis*, Pringsh. (Schmidle, '00 A, p. 160; Nordstedt, '78, p. 23; Lemmermann, '05, p. 633; Gutwinski and Chmielewski, '06, p. 2); *C. scutata*, Bréb. (Turner, '92, p. 164; ? Wille, '84, p. 26; Schmidle, '00 A, p. 160; West and West, '02 B, p. 125; Borge, '96, p. 3; Dickie, '81, p. 123); *C. soluta*, Pringsh. (Lagerheim, '93, p. 155; West and West, '02 B, p. 125).

IX. *Chrodepideae* (see pp. 242, 243).

The genera best represented are thus *Microspora*, *Stigeoclonium*, *Ulothrix*, and, to a less extent, *Aphanochaete*; *Coleochaete* is evidently quite a common form, and I also met with it fairly frequently in Ceylon. It is interesting that there is practically no record of a *Prasiola* from the tropics. This genus is, however, a terrestrial form, and as such is probably excluded by the same factors as oppose the development of subaerial green forms

generally (p. 239). *Draparnaldia* has as yet only been recorded from the Sandwich Islands and Ecuador; it was not observed in Ceylon.

Although a considerable number of different species and genera of Ulotrichales are thus known to occur in the tropics, the number of actual records is not very great, and I am inclined to think that this group does after all take rather a back place in these regions. Possibly this may again be a result of the difficulties of respiration in the tropics, although there is nothing to show that these forms favour specially well-aerated tropical waters. Some species of *Ulothrix*, even in our parts of the world, are known to flourish best in flowing water, and it seems very possible that forms, which can get on satisfactorily in stagnant temperate waters, may be unsuccessful in similar tropical habitats. After all, we know as good as nothing at present as to the influence of the amount of dissolved oxygen in the water on the various freshwater algal genera.

(v) The Zygnemaceae in the Tropics.

Probably no feature of the freshwater algal flora of the tropics has played such a part in producing the universal impression of similarity with that of temperate regions as the excessive abundance of *Spirogyra*. This genus is indeed even more abundant than it is with us, and yet careful examination of the available data shows that the tropical *Spirogyras* have certain characteristic features, which are sufficiently striking. *Spirogyra* is of course an essentially stagnant-water form, and this, I think, is one reason for its immense success in the tropics. As pointed out above (p. 246) *Spirogyra* and *Pithophora* (together with a certain number of species of *Oedogonium*) are the only forms with really broad filaments found in the ordinary tropical freshwaters, and I am inclined to associate this with the evident small demand for aerated water made by *Spirogyra*. Moreover, the narrower species of this genus are even rather scarce in the tropics, and the broad ones are dominant (see the measurements given in the enumeration below). In relation to this point we have also to notice that species of *Spirogyra* with a single spiral in their cells are scarce (twenty records), and that the majority of forms have two, three, or more such chloroplasts (sixty-two records). Lastly, only five (viz. *S. inflata*, *S. quadrata*, *S. tenuissima*, *S. Grevilleana*, and *S. insignis*) of the forms hitherto recorded (six records) have infolded transverse walls, the latter almost invariably being simple.[1] The same observations were made on the abundant material of *Spirogyra* collected in Ceylon; broad forms with many spirals were preponderant, and no case of infolded end-walls has been met with up to the present.

[1] Amongst the *Spirogyras* enumerated by De Toni ('89, p. 743 et seq.) there are thirty species with a single chloroplast and forty-three with two or more in their cells; amongst the species known from the tropics there are nine with a single spiral, and twenty-nine with two or more spirals. The difference in number is obvious. De Toni has twenty-two species with infolded walls as compared with fifty-one with simple end-walls. In the tropical records the relation is 5 : 33.

In the following enumeration of tropical *Spirogyras*[1], the species are arranged according to the number of chloroplasts in the cells in order to bring out the above point. The average width of the filaments in each species is added in brackets:—

(i) Cells with a single spiral:—

1. *Sp. arcta* (Ag.), Kütz. (33–36 μ):—West and West, '96, p. 377.
2. *S. cateniformis* (Hass.), Kütz. (26–27·5 μ):—Borge, '03, p. 280.
3. *S. condensata* (Vauch.), Kütz. (48–62 μ):—? Möbius, '90, p. 1069.
4. *S. Goetzei*, Schmidle (22–24 μ):—Schmidle, '02 B, p. 251.
5. *S. gracilis*, Kütz. (17·5–19 μ):—West and West, '02 A, p. 162; Lagerheim, '93, p. 162 (var. *β abyssinica*, Lagerh., 10 μ diam.).
6. *S. inflata* (Vauch.), Rabh. (18–19·5 μ):—Borge, '03, p. 279.
7. *S. longata*, Kütz. (24–27 μ):—Schmidle, '00 A, p. 161; Schmidle, '02 B, p. 251; ? Dickie, '81, p. 125; Zeller, '73, p. 185; Prain, '05, p. 328 (f. *elongata*, Kütz.); Lagerheim, '90, p. 7 (f. *elongata*, Kütz.); Lagerheim, '90, p. 15; Möbius, '89, p. 314 (f. *elongata*).
8. *S. quinina*, Kütz. (25·5–43 μ):—Zeller, '73, p. 185; Borge, '06, p. 6 (sub *S. porticalis* (Müll.), Cleve).
9. *S. tenuissima* (Hass.), Kütz. (8 μ):—Lagerheim, '90, p. 7 (var. *plena*, Lagerh.).

(ii) Cells with two or three spirals:—

1. *S. adnata*, Kütz.[2] (40–45 μ):—Zeller, '73, p. 185. (2 spirals!)
2. *S. angelensis*, Welw. MS. (49–63 μ):—West and West, '97 A, p. 41. (2, rarely 3 spirals!)
3. *S. decimina*, Kütz. (32–50 μ):—West and West, '95, p. 42; West and West, '02 A, p. 161; West and West, '02 B, p. 133; Zeller, '73, p. 185; Zanardini, '72, p. 152. (2, rarely 3 spirals!)
4. *S. dubia*, Kütz. (43–50 μ):—West and West, '96, p. 378; Dickie, '81, p. 125 (var. *longiarticulata*). (2 or 3 spirals!)
5. *S. Grevilleana* (Hass.), Kütz. (20–33 μ):—Borge, '03, p. 280. (1 or 2 spirals!)
6. *S. irregularis*, Näg.[2] (32–36 μ):—Zeller, '73, p. 185. (2 or 3 spirals!)
7. *S. quadrata* (Hass.), Petit (27–33 μ):—Schmidle, '02 B, p. 251; Lagerheim, '90, p. 15. (1 or 2 spirals!)
8. *S. rupestris*, Schmidle (33 μ):—Schmidle, '00 A, p. 170; Schmidle, '00 B, p. 18. (2 or 3 spirals!)
9. *S. Schmidtii*, W. and G. S. West (31–35 μ):—West and West, '02 A, p. 161. (2 or 3 spirals!)
10. *S. Welwitschii*, W. and G. S. West (65–75 μ):—West and West, '97 A, p. 41. (2, rarely 3 spirals!)

(iii) Cells with three or often a larger number of spirals:—

1. *S. crassa*, Kütz. (120–150 μ):—Zeller, '73, p. 185; Gutwinski, '02 A, p. 578 (var. *maxima* (Hass.), Hansg.); Prain, '05, p. 329 (sub *S. Heeriana*, Näg.).

[1] Undetermined sterile species are not taken into account.

[2] A certain amount of doubt attaches to these determinations.

2. *S. cylindrospora*, W. and G. S. West (70–77 μ):—West and West, '97 A, p. 42.

3. *S. Füllebornei*, Schmidle (40–42 μ):—Schmidle, '03 A, p. 76.

4. *S. Holstii*, Hieron. (55–65 μ):—Hieronymus, '95, p. 21.

5. *S. insignis* (Hass.), Kütz. (39–42 μ):—Möbius, '89, p. 314.

6. *S. jugalis*, Kütz. (72–98 μ):—Zeller, '73, p. 185.

7. *S. lineata*, Suring. (50–65 μ):—Henriques, '86, p. 218 (f. *gracilior*).

8. *S. majuscula*, Kütz. (54–62 μ):—Zeller, '73, p. 185; De Wildeman, '97, p. 84 (var. *minor*, Wittr. et Nordst.); De Wildeman, '00, p. 116 (var. *minor*, Wittr. et Nordst.).

9. *S. Malmeana*, Hirn (76–91 μ):—Schmidle, '01 A, p. 46 (var. *minor?*); Borge, '03, p. 281.

10. *S. maxima*, Wittr. (104–117 μ):—Borge, '99, p. 10; Borge, '03, p. 281.

11. *S. neglecta*, Kütz. (53–65 μ):—West and West, '02 A, p. 161; West and West, '02 B, p. 133; West and West, '96, p. 378 (var. *ternata* (Ripart)); West and West, '97 A, p. 41 (var. *ternata*); West and West, '99, p. 281 (var. *ternata*).

12. *S. nitida* (Dillw.), Link. (60–78 μ):—? Möbius, '93, p. 123; Schmidle, '95, p. 298; De Wildeman, '00, p. 114; ? Möbius, '90, p. 1068; Prain, '05, p. 328; Zeller, '73, p. 185; Martens, '66, p. 21; Askenasy, '89 (sub *S. princeps* (Vauch.), Cleve).

13. *S. pallida*, Dickie ? (30 μ):—Dickie, '80, p. 281 (see also Hieronymus, '95, p. 21).

14. *S. paraguayensis*, Borge (41–45 μ):—Borge, '03, p. 280.

15. *S. Reinhardii*, Chmiel. (108–117 μ):—Borge, '03, p. 281.

16. *S. rivularis*, Rabh. (36–40 μ):—Schmidle, '02 A, p. 65; ? Möbius, '88, p. 242.

17. *S. setiformis* (Roth), Kütz. (85–110 μ):—West and West, '96, p. 377; ? Möbius, '93, p. 123; De Wildeman, '00, p. 115.

18. *S. tropica*, Kütz. (70–90 μ?):—? Möbius, '88, p. 242; Dickie, '81, p. 125; Martens, '70, p. 298; ? Möbius, '92, p. 24; Zeller, '73, p. 185; Zeller, '76, p. 427.

19. *S. variabilis*, De Wild. (80–88 μ):—De Wildeman, '97, p. 83; De Wildeman, '00, p. 115.

Although this list shows to some extent the preponderance of broad forms with several chloroplasts to the cells, it is of course merely an enumeration of species and records, and, as I have had frequent occasion to point out already, it gives us no idea of the relative abundance of individuals. Still, it gives some support to the view that, as observed in Ceylon, the tropics may in general have a relatively larger number of broad *Spirogyras* than our parts of the world. It is certainly striking in how many of the tropical floras we find almost only broad forms mentioned (cf., for instance, De Wildeman, '00, West and West, '97 A and '02 B). It may be that further observation may show that the above inference is not correct, but the matter is certainly worthy of attention.

There is little that is especially noticeable regarding the distribution of *Spirogyra* in the tropics, and it is probable that most of the species will

be found to have a fairly wide range. It is noticeable that, although the algal flora has received considerable attention, only sterile *Spirogyras* have as yet been recorded from the Sandwich Islands. There are no records at all in Volken's algal collections from the Carolines, although they are so scanty that not much weight can be attached to this point (Schmidle, '01 B). Schmidle ('97 B) does not record any *Spirogyras* from the tropical parts of Polynesia, but from the Samoa Islands (Schmidle, '97 E) sterile species again are known. The literature offers too few data as to habitat to enable one to say anything of general application on this subject. In Ceylon, *Spirogyra* was found most abundantly in smaller, absolutely stagnant pieces of water (small ditches, rice-fields, &c.), and generally occurred in localities where it was more or less shaded either by a dense growth of aquatic Phanerogams or by the surrounding terrestrial vegetation.

So little is known about the significance of the infolded transverse walls found in many of the narrower species of *Spirogyra* that we are not in a position to understand the reasons for the scarcity of species exhibiting this phenomenon in tropical waters. The infolding is usually looked upon as a mechanism for the disjunction of the cells of a filament, but from this point of view its scarcity in the tropics is inexplicable. Since it is mostly found in quite narrow forms, the rarity of these latter may be responsible, although that does not seem very likely.

In comparison to *Spirogyra* the other genera of Zygnemaceae play a very small part in the tropics; that is, however, the same relation as obtains in our flora. There are records of a considerable number of species of *Mougeotia* and *Zygnema* (incl. *Zygogonium*), also *Gonatonema* (*G. tropicum*, W. and G. S. West) and *Sirogonium* (*S. sticticum*, Kütz.). All these four genera have also been met with in Ceylon. In addition to this we have the two monotypic genera *Temnogametum*, W. and G. S. West, and *Pyxispora*, W. and G. S. West, from Africa (West and West, '97 A). *Debarya*, Wittr., has not yet been recorded from the tropics.

(vi) The Desmids of the Tropics.

Diverse as the Desmid-flora of temperate waters is, it appears to be excelled in the tropics. There is quite an extensive literature dealing with floristic records of tropical Desmids, and I must refer to the same for details on this point. One cannot say that a group so abundant in our flora finds more favourable conditions of existence in the tropics, but it does appear that along certain lines evolution of form in the Desmids has surpassed itself in tropical waters.

The only object I have in view here in dealing with tropical Desmids is to point out the important part which filamentous forms play. In our flora filamentous Desmids are distinctly rare, *Hyalotheca dissiliens*, Bréb., and *Gymnozyga moniliformis*, Ehrenb., perhaps being the two commonest.

But examination of certain types of tropical waters (in Ceylon, especially small stagnant ditches and rock-pools) shows a wealth of such forms, which, although not very great in number of species, makes up for it by the large number of individuals. In such waters we also find many forms making attempts at filament-formation, which ordinarily exist as independent individuals (*Micrasterias foliacea*, Bail., species of *Pleurotaenium*, *Triploceras*, *Euastrum*, &c.). The relative excess of filamentous Desmids in tropical waters comes out very plainly in comparing pools with a rich Desmid-flora from the lowlands and uplands (from around Nuwara Elija, alt. 6,000 feet and more) of Ceylon. The upland pools (in which the conditions of existence are semi-temperate) were found to be just as poor in filamentous forms as the waters of temperate regions. I have elsewhere [1] suggested that we may again find an explanation for this phenomenon in the relative aeration of the water, the presence of little dissolved oxygen appearing to encourage filament-formation. This is a suggestion [2] which must be left to experiment for verification, but it would certainly explain the relative abundance of filamentous Desmids in tropical waters. The same theory also accounts for the abundance of filamentous Diatoms in hot springs (cf. p. 253).

In turning our attention to the literature with reference to the point just discussed, I know of no more striking example to illustrate it than the records in Borge's paper on Australian freshwater Chlorophyceae (Borge, '96). The Algae enumerated in this paper are partly tropical and partly extra-tropical; of the sixteen species of filamentous Desmids recorded, thirteen are from tropical habitats, six only from extra-tropical localities. In further illustration of the same feature we may notice the number of species of filamentous Desmids in some of the more complete tropical algal floras, viz. Borge, '03 (sixteen species); De Wildeman, '00 (eleven species and numerous varieties); Joshua, '86 (thirteen species); Nordstedt, '80 (seven species and numerous varieties); Raciborski '95 (twelve species); Schmidle, '03 A (seven species); Turner, '92 (twenty-six species); West and West, '02 B (twenty-one species). It is, of course, possible that there are regions of the tropics in which filamentous Desmids are not as important as they no doubt are in certain waters in Ceylon, but I think it more probable that the lack of records in many of the floras is due to the material not having been collected from habitats in which these Desmids flourish. Small collections of stagnant water are apparently the most favourable; this is also shown by the large number of filamentous forms found amongst the Desmids, which live in the bladders of tropical species of *Utricularia* (cf. especially Raciborski, '95, p. 30; Nordstedt, '80).

[1] Proc. Roy. Soc., Ser. B, vol. lxxix, 1907.

[2] This theory is based on Senn's observations on colonial Protococcales (Bot. Zeitung, Bd. lvii, 1899, p. 97).

(vii) The Oedogoniaceae in the Tropics.

It has already been pointed out above that the species of *Oedogonium* found in the freshwaters of the tropics are prevalently narrow forms. This fact is very striking if we examine the literature. Thus the following species have filaments, whose diameter is 10 μ or less[1]:—

A. 1. *Oedogonium angustissimum*, W. and G. S. West (1·8–2 μ):—West and West, '97 A, p. 6.
2. „ *Aster*, Wittr. (7·5–8 μ):—West and West, '02 B, p. 128.
3. „ *cryptoporum*, Wittr. (5–7·5 μ):—West and West, '02 A, p. 158; West and West, '97 A, p. 4.
4. „ *cryptoporum*, Wittr., var. *vulgare*, Wittr. (6·5–10 μ):—West and West, '02 B, p. 127.
5. „ *elegans*, W. and G. S. West (6–8·5 μ):—West and West, '02 B, p. 128.
6. „ *excisum*, Wittr. et Lund (3·5–5·5 μ):—Nordstedt, '80, p. 13.
7. „ *Franklinianum*, Wittr. (8–12 μ):—Möbius, '93, p. 118; De Wildeman, '00, p. 51.
8. „ *gracillimum*, Wittr. et Lund (6·5–7 μ):—West and West, '97 A, p. 4 (f. *major*).
9. „ *Howardii*, G. S. West (7·5–11 μ):—West, '04, p. 281.
10. „ *Itzigsohnii*, De Bary (4·8–5·7 μ):—West and West, '97 A, p. 4 (var. *minor*).
11. „ *longicolle*, Nordst. (4–6 μ):—West and West, '02 B, p. 128; Nordstedt, '78, p. 20; Lemmermann, '05, p. 633.
12. „ *longicolle*, Nordst., var. *β. senegalensis*, Nordst. (5–7·5 μ):—Nordstedt, '80, p. 13; Lagerheim, '93, p. 155; West and West, '02 B, p. 128; Schmidle, '95, p. 294.
13. „ *mammiferum*, Wittr. (7·5 μ):—West and West, '97 A, p. 5 (sub *O. huillense*, W. and G. S. West).
14. „ *obsoletum*, Wittr. (9–15 μ):—Lemmermann, '05, p. 633.
15. „ *Petri*, Wittr. (5–8 μ):—West and West, '02 B, p. 127.
16. „ *platygynum*, Wittr. (5–5·7 μ):—West and West, '02 B, p. 127.
17. „ *pusillum*, Kirchner (3–8 μ):—Lemmermann, '06, p. 160; Lagerheim, '93, p. 155 (sub *O. africanum*, Lagerh.).
18. „ *pyrulum*, Wittr. (8–11·5 μ):—West and West, '02 B, p. 127.
19. „ *reticulatum*, W. and G. S. West (7·5–8 μ):—West and West, '02 B, p. 129.
20. „ *Rothii* (Le Cl.), Pringsh. (8·5–10·5 μ):—West and West, '97 A, p. 5 (f. *major*); Zeller, '73, p. 188.
21. „ *rugulosum*, Nordst. (4·5–8 μ):—Lagerheim, '93, p. 155.

[1] This list has been revised according to Hirn's monograph (Act. Soc. Scient. Fennicae, t. xxvii, No. 1, 1900; and t. xxxiv, No. 3, 1906). The measurements are taken from the actual records, wherever they were given; otherwise from Hirn.

22. *Oedogonium Sancti Thomae*, Wittr. et Cleve (7–15 μ):—Wittrock, '78, p. 141.
23. „ *tapeinosporum*, Wittr. (2·7–5 μ):—Wittrock, '78, p. 140.
24. „ *tapeinosporum*, Wittr., var. *angolense*, W. and G. S. West (3·8–4·5 μ):—West and West, '97 A, p. 5.
25. „ *Warmingianum*, Wittr. (8–9 μ):—Wittrock, '78, p. 140.

B. In the following species the diameter is 20 μ or less:—

1. *Oedogonium acrosporum*, De By. (13–21 μ):—Wille, '84, p. 25; Lagerheim, '93, p. 155.
2. „ *acrosporum*, De By., var. *majusculum*, Nordst. (14–21 μ):—Nordstedt, '78, p. 21; Lemmermann, '05, p. 633.
3. „ *areolatum*, Lagerh. (17–21 μ):—Lagerheim, '90, p. 2 and 11.
4. „ *brasiliense*, Borge (16–22 μ):—Borge, '99, p. 4.
5. „ *Braunii*, Kütz. (13–15 μ):—Zeller, '73, p. 188; Lagerheim, '90, p. 2.
6. „ *Cleveanum*, Wittr. (14–25 μ):—Schmidle, '01 B, p. 344.
7. „ *crispum* (Hass.), Wittr. (12–16 μ):—Turner, '92, p. 163; Wittrock, '78, p. 133.
8. „ *crispum* (Hass.), Wittr., var. *havaiense*, Nordst. (12–16 μ):—Nordstedt, '78, p. 20; Lemmermann, '05, p. 633.
9. „ *crispum* (Hass.), Wittr., var. *Uruguayense*, Magn. et Wille (13·5–15 μ):—West and West, '97 A, p. 4.
10. „ *dictyosporum*, Wittr. (11–16 μ):—Wittrock, '78, p. 134.
11. „ *foveolatum*, Wittr. (14–23 μ):—Wittrock, '78, p. 133.
12. „ *globosum*, Nordst. (10–14 μ):—Nordstedt, '78, p. 20; Lemmermann, '05, p. 633; Wille, '03, p. 93.
13. „ *laetevirens*, Wittr. (12–13 μ):—Zeller, '76, p. 427; Wittrock, '78, p. 135.
14. „ *londinense*, Wittr. (12·5–13·5 μ):—West and West, '97 A, p. 6.
15. „ *obtruncatum*, Wittr., var. *ellipsoideum*, Wittr. (17–23 μ):—Wittrock, '78, p. 141.
16. „ *Paulense*, Nordst. et Hirn (10–14 μ):—Schmidle, '01 B, p. 344.
17. „ *Pithophorae*, Wittr. (10–13 μ):—Wittrock, '78, p. 141.
18. „ *pluviale*, Nordst. (19–25 μ):—West and West, '02 A, p. 160.
19. „ *Pringsheimii*, Cram. (10–15 μ):—Nordstedt, '78, p. 21 (sub *O. pachydermatosporum*, Nordst.).
20. „ *Pringsheimii*, Cram., var. *Nordstedtii*, Wittr. (12–14 μ):—Schmidle, '97 E, p. 260 (sub *O. Pringsheimii*, var. *varians*, Nordst.).
21. „ *punctatum*, Wittr. (18–22 μ):—Wittrock, '78, p. 142.
22. „ *Sodiroanum*, Lagerh. (20 μ):—Lagerheim, '90, p. 2.
23. „ *stellatum*, Wittr. (13·5–17 μ):—West and West, '02 B, p. 128.
24. „ *undulatum* (Bréb.), A. Br. (14–16 μ):—Borge, '99, p. 4; Turner, '92, p. 163; Borge, '96, p. 4.

25. *Oedogonium undulatum* (Bréb.), A. Br., var. *senegalense*, Nordst. (14–20 μ):—Nordstedt, '80, p. 13.
26. „ *Wehwitschii*, W. and G. S. West (17–24 μ):—West and West, '97 A, p. 5.

C. In the following species the diameter is 30 μ or less:—
1. *Oedogonium cyathigerum*, Wittr., f. *ornatum* (Nordst.), Hirn (21–30 μ):—Wittrock, '78, p. 134.
2. „ *cyathigerum*, var. *hormosporum* (W. and G. S. West), Hirn (22–30 μ):—West and West, '97 A, p. 5 (sub *O. hormosporum*, W. and G. S. West; in stagnis pr. Catumba); Schmidle, '03 A, p. 86 (ditch near Langenburg).
3. „ *indicum*, Hirn (20–25 μ):—Schmidle, '00 A, p. 160 (no locality given).
4. „ *oboviforme*, Wittr. (21–32 μ):—Wittrock, '78, p. 140.
5. „ *plagiostomum*, Wittr., var. *gracilius*, Wittr. (22–25 μ):—Wittrock, '78, p. 142.
6. „ *spirale*, Hirn, var. *acutum*, W. and G. S. West (22–25 μ):—West and West, '02 B, p. 128 (rice-fields).
7. „ *Wolleanum*, Wittr. (21–30 μ):—Schmidle, '00 A, p. 160 (no locality given).

D. The diameter is below 40 μ in:—
1. *Oedogonium dioicum*, Carter (31–35 μ):—West and West, '02 A, p. 159 (stagnant water).
2. „ *Landsboroughi* (Hass.), Wittr. (31–40 μ):—Schmidle, '00 A, p. 160 (no locality given); Zeller, '73, p. 188.
3. „ *mexicanum*, Wittr. (34–40 μ):—Wittrock, '78, p. 138.

E. The diameter is over 40 μ in:—
1. *Oedogonium fabulosum*, Hirn, var. *maximum*, W. and G. S. West (83–93 μ):—West and West, '02 A, p. 159 (sub *O. maximum*, W. and G. S. West; stagnant water).
2. „ *Kurzii*, Zeller (44–52 μ):—Zeller, '73, p. 189 (marsh).
3. „ *suboctangulare*, W. and G. S. West (50–54 μ):—West and West, '02 B, p. 127 (rice-fields).

The list shows the great prevalence of narrow forms; by far the majority (viz. 51) are less than 20 μ, and only thirteen species exceed these dimensions. I have above indicated that I am inclined to explain this as a result of the small amount of dissolved oxygen in the water. In all the forms whose filaments are more than 20 μ in width data as to habitat have been added, where these are available. These data, however, do not show that the broad forms in question are found in anything but stagnant water, so that it does not appear that they have got over the difficulties of poor aeration by frequenting special habitats. *O. suboctangulare*, to judge by the figures (West and West, '02 B, Pl. XVII, Figs. 1, 2), has exceptionally thin walls, and the same is true to a less extent of *O. fabulosum*, var.

maximum (cf. West and West, '02 A, Pl. IV, Figs. 39–41). Possibly the success of these few broad forms lies in this feature. It is, however, most requisite that the tropical habitats of the broad species of *Oedogonium* should be carefully studied.

The genus *Bulbochaete* is not very well represented, but it is a form which may fairly easily escape notice. There is nothing, as far as I am aware, of particular interest attaching to its occurrence in tropical waters.

5. The Freshwater Florideae of the Tropics.

Since the Florideae, like the Cyanophyceae, are provided with a protective pigment (in the form of phycoerythrin and its modifications), we might expect this group to be a successful one in the tropics. It is, however, an essentially marine group, and has but few freshwater representatives, all of which are not very common in our parts of the world. In the tropics, on the other hand, they seem to be not at all uncommon, as shown by the following enumeration of records:—

1. *Batrachospermum angolense*, W. and G. S. West (West and West, '97 A, p. 2; West and West, '02 B, p. 125); *B. Bohneri*, Schmidle (Schmidle, '99, p. 2; Schmidle, '02 A, p. 67); *B. bornense*, Zanardini (Zanardini, '72, p. 146); *B. gracillimum*, W. and G. S. West (West and West, '97 A, p. 2); *B. huillense*, Welw. MS. (West and West, '97 A, p. 3); *B. moniliforme*, Roth (Gutwinski and Chmielewski, '06, p. 2; Möbius, '90, p. 1070; Martens, '70, p. 299; Möbius, '92, p. 22; Zeller, '73, p. 192); *B. moniliforme*, vars. *proliferum* and *nodiflorum* (Dickie, '81, p. 33); *B. nigrescens*, W. and G. S. West (West and West, '97 A, p. 2); *B. Puiggarianum*, Grun. (Möbius, '92, p. 22); *B. Schwackeanum*, Möbius (Möbius, '92, p. 18); *B. vagum*, Ag. (Möbius, '92, p. 22); *B. villosum*, Zanardini (Zanardini, '72, p. 147).

2. *Chantransia chalybea*, Fr. (Möbius, '95, p. 174); *C. pulvinata*, Schmidle (Schmidle, '00 A, p. 188; Schmidle, '00 B, p. 79); *C. roseola*, Zeller (Zeller, '73, p. 192)

3. *Compsopogon chalybeus*, Kütz. (Möbius, '88, p. 222; Möbius, '90, p. 1070; Zeller, '76, p. 427); *C. fuscatus*, Zanardini (Zanardini, '72, p. 149); *C. leptoclados* Mont. (Dickie, '81, p. 123; Martens, '71, p. 144).

4. *Hildenbrandtia angolensis*, Welw. MS (West and West, '97 A, p. 3); *H. rivularis* (Liebm.), J. Ag. (West and West, '97 A, p. 3; Hariot, '91, p. 1220).

5. *Thorea flagelliformis*, Zanardini (Zanardini, '72, p. 148); *T. Gaudichaudii*, Ag. (Schmidle, '00 D, p. 24); *T. ramosissima*, Bory (De Wildeman, '00, p. 397); *T. Traili*, Dickie (Dickie, '81, p. 123).

Lemanea does not appear to have been recorded from the tropics up to the present.

As in our parts of the world, the freshwater Florideae of the tropics are found prevalently in running water, and appear to occur most commonly in mountainous regions. I have not been able to settle whether they are ever found in the actual lowlands of the tropics.

SUMMARY.

The more important conclusions or suggestions brought forward in the present paper may be briefly summarized as follows:—

(i) There is some evidence to show that in the damp tropics there is always a very extensive subaerial algal covering, equal to or perhaps even surpassing that examined by the author in Ceylon.

(ii) The subaerial algal growth in Ceylon consists almost entirely of Cyanophyceae, and there is evidence in the literature that in other parts of the tropics this group is equally predominant in the subaerial algal flora. There are not very many records of green subaerial forms in the literature (see however iv), and it is probable that they play a very small part in the tropics.

(iii) The Cyanophyceae are probably an essentially tropical group, and it is not impossible that they may be the descendants of primitive algal forms, which existed in earlier periods under conditions analogous to those found in the damp tropics at the present day.

(iv) *Trentepohlia* is the only genus of green Algae that is really successful in the subaerial flora (apart from other members of the Chroolepideae, which have become epiphyllous, and from the parasitic genera *Phyllosiphon* and *Phytophysa*.)

(v) In the submerged algal flora of tropical freshwaters the Cyanophyceae also constitute a very important element, though not as preponderant as in the subaerial flora. They are equally important in the Plankton.

(vi) The freshwater algal flora of the tropics is composed of forms in which narrow filaments are much more abundant than broad ones, a fact which is brought into relation with the small amount of dissolved oxygen in the water. Exceptions are constituted by *Pithophora* and *Spirogyra*, especially by the latter.

(vii) *Cladophora* and *Rhizoclonium* are very poorly represented in tropical freshwaters in comparison to their abundant occurrence in our parts, and there is good evidence to show that in most cases they favour well-aerated (e. g. running) water. The chief representative of Cladophoraceae in the tropics is *Pithophora*, which appears to be an essentially tropical genus.

(viii) *Vaucheria* is very rare in the tropics. This refers especially to the aquatic species, but the terrestrial ones are not much commoner. *Botrydium* is equally rare. It is possible that the peculiar assimilatory process in these two forms is responsible (the same applies to the Confervales).

(ix) The Confervales are not very well represented, the most abundant form being *Ophiocytium*. *Conferva* is not well represented, and tends to be restricted to well-aerated water.

(x) In the case of the Ulotrichales it is not quite evident at present

whether they attain an adequate development in tropical freshwaters, but there are slight indications to the contrary.

(xi) The genus *Spirogyra* is even more abundant in the tropics than it is with us. The species so far recorded are mainly broad forms with two or many chloroplasts in their cells. Forms with infolded end-walls are strikingly rare. The other genera of Zygnemaceae are not very abundantly represented.

(xii) There is a marked filamentous tendency amongst the Desmids of tropical waters (especially in certain kinds of stagnant water), and it is suggested that this may again be due to the poor aeration of the water.

(xiii) The genus *Oedogonium* is very abundant in the tropics, but is mainly represented by species with narrow filaments.

(xiv) Freshwater Florideae appear to be not at all uncommon in the tropics.

In conclusion, I should like to mention that I am fully aware of the speculative basis of a great part of the matter contained in this paper; preliminary experiments are, however, already in progress with a view to testing the accuracy of many of the suggestions. The investigation is in too early a stage at present to admit of any communication on the subject. I should also state that I am indebted to the Government Grant Committee of the Royal Society for the grant which made it possible to investigate the freshwater algal flora of Ceylon—an investigation which has been the chief stimulus for the present paper.

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February 18, 1907.

BIBLIOGRAPHY.[1]

1. ASKENASY ('89): E. Askenasy, Algen, mit Unterstützung der Herrn E. Bornet, A. Grunow, P. Harlot, M. Möbius, O. Nordstedt, in Die Forschungsreise S.M.S. 'Gazelle' in den Jahren 1874 bis 1876. IV. Teil: Botanik. Berlin, 1889, pp. 1–58 (only partly tropical).
2. BOERGESEN ('90): F. Boergesen, Desmidieae, in 'Symbolae ad floram Brasiliae centralis cognoscendam'. Vid. Meddelels. fra d. Nat. Foren. i. Kjøbenhavn, 1890, pp. 24–53.

[1] I have already pointed out that this bibliography, though great pains have been taken to include all the important papers dealing with the tropical freshwater and subaerial algal flora, is not exhaustive. Very few of the older papers are included, and of recent ones many minor contributions, not bearing on the main points of the present paper, have been omitted. I have also not taken into account the records in De Toni's 'Sylloge,' since a considerable number would have been duplicates of those already given. Nor have any *exsiccata* been considered for the same reasons. Messrs. Wittrock, Nordstedt and Lagerheim's 'Algae aquae dulcis exsiccatae' do indeed contain a certain number of records of tropical forms, which, as far as I am aware, are not published elsewhere, but reference to the recent general index (published, Lund, 1903) will show them at a glance.

Certain papers marked with a star were not considered in all points sufficiently distinct or precise to be taken into account in all of the lists compiled in the course of my paper; omission of data from them in certain cases must not therefore be attributed to negligence on my part.

3. Bohlin ('97): K. Bohlin, Die Algen der ersten Regnell'schen Expedition. I.: Protococcoideen. Bih. Sv. Vet.-Ak. Handl., xxiii, Afd. III, No. 7, 1897.
4. Borge ('96): O. Borge, Australische Süsswasserchlorophyceen. Bih. K. Sv. Vet.-Ak. Handl., xxii, Afd. III, 1896, No. 9 (only partly tropical).
5. —— ('99): O. Borge, Ueber tropische und subtropische Süsswasser-Chlorophyceen. Bih. K. Sv. Vet.-Ak. Handl., xxiv, Afd. III, 1899, No. 12 (only partly tropical).
6. —— ('03): O Borge, Die Algen der ersten Regnell'schen Expedition. II.: Desmidiaceen, III.: Zygnemaceen und Mesocarpaceen. Arkiv f. Bot. Stockholm, 1903, Bd. i, p. 71 et seq. (only partly tropical).
7. —— ('06): O. Borge, Algen aus Argentina und Bolivia. Arkiv för Botanik, K. Sv. Vet.-Akad. i Stockholm, Bd. vi, 1906, No. 4 (only partly tropical).
8. Borzi ('92): A. Borzi, Alghe d'acqua dolce della Papuasia raccolte su cranii umani dissepolti. Nuovo Notarisia, Ser. 3, 1892, p. 35 et seq.
9. Cohn ('79): F. Cohn, Desmidiaceae Bongoenses. Festschr. Naturf. Ges. Halle a/S., 1879, p. 261 et seq.
10. Cunningham ('80): D. D. Cunningham, On *Mycoidea parasitica*, a new genus of parasitic Algae. Trans. Linn. Soc., Ser. 2, vol. i, 1880.
11. De Toni ('89): G. B. De Toni, Sylloge Algarum omnium hucusque cognitarum. Vol. i.: Chlorophyceae. Patavii, 1889.
12. ——— ('91): G. B. De Toni, Ueber eine neue *Tetrapedia*-Art aus Afrika. Hedwigia, xxx, 1891, pp. 194-6.
13. ——— ('92): G. B. De Toni, Algae abyssinicae a cl. Prof. O. Penzig collectae. Malpighia, v. 1891-92, pp. 261-73.
14. De Toni and Saccardo ('90): G. B. De Toni and F. Saccardo, Revisione di alcuni generi di chlorophycee epifite. Nuovo Notarisia, 1890.
15. De Wildeman ('88): É. De Wildeman, Sur quelques formes du genre *Trentepohlia*. Comptes-Rendus Soc. Roy. Bot. de Belgique, xxvii, 1888, pp. 178-82 (also loc. cit. xxviii, 1889, pp. 67-70).
16. ——— ('89): É. De Wildeman, Quelques mots sur la flore algologique du Congo. Comptes-Rendus, Soc. Roy. Bot. de Belgique, xxviii, 1889, pp. 6-10.
17. ——— ('91): É. De Wildeman, Les *Trentepohlia* des Indes Néerlandaises. Ann. Jard. Bot. Buitenzorg, ix, 1891, pp. 127-42.
18. ——— ('94): É. De Wildeman, Notes sur quelques espèces du genre *Trentepohlia* (Martius). Annales (Mémoires) Soc. belge de Microscopie, xviii, 1894, p. 5 et seq. (also loc. cit., xxi, 1897, p. 95 et seq.).
19. ——— ('97): É. De Wildeman, Algues rapportées par M. J. Massart d'un voyage aux Indes Néerlandaises. Ann. Jard. Bot. Buitenzorg, 1re Supplém., Leiden, 1897, p. 32 et seq.
20. ——— ('00): É. De Wildeman, Les Algues de la flore de Buitenzorg. Leiden, 1900. (Cf. also Prodrome de la flore algologique des Indes Néerlandaises, Batavii, 1897-99).
21. Dickie (75*): G. Dickie, On the Algae of Mauritius. Journ. Linn. Soc. Bot., vol. xiv, 1875, p. 190 et seq.
22. —— ('80): G. Dickie, Notes on Algae from Lake Nyassa, E. Africa. Journ. Linn. Soc. Bot., vol. xvii, 1880, p. 281 et seq.
23. —— ('81*): G. Dickie, Notes on Algae from the Amazons and its Tributaries. Journ. Linn. Soc. Bot., vol. xviii, 1881, p. 123 et seq.
24. Gomont ('02): M. Gomont, Myxophyceae hormogoneae, in J. Schmidt, Flora of Koh Chang. Botanisk Tidsskrift, xxiv, 1902, p. 202 et seq.
25. Grunow ('65): A. Grunow, Ueber die von Herrn Gersterberger in Rabenhorst's Decaden ausgegebenen Süsswasser-Diatomaceen und Desmidiaceen von der Insel von Banka, etc. Rabenhorst, Beitr. z. Kenntn. d. Verbreit. d. Algen, II. Heft, 1865 (see also Hedwigia, iv, 1865, p. 124 et seq.).
26. ——— ('67): A. Grunow, Algen, in Reise seiner Majestät Fregatte 'Novara' um die Erde, Botan. Theil, I. Bd., Wien, 1867, p. 1 et seq. (only partly tropical).
27. Gutwinski ('02 A): R. Gutwinski, De Algis a Dr. M. Raciborski anno 1899 in insula Java collectis. Bull. Internat. Ac. Sci. Cracovie., No. 9, November, 1902, p. 575 et seq.
28. ——— ('02 B): R. Gutwinski, Additamenta ad floram algarum Indiae Batavorum

cognoscendam. Algae a cl. Dre. M. Raciborski in montibus vulcanis Krakatau et Slamat anno 1897 collectae. Verh. u. Sitzber. Ak. Wiss. Krakau, xxxix, 1902, p. 287 et seq. (not seen).

29. GUTWINSKI and CHMIELEWSKI ('06): R. Gutwinski and Z. Chmielewski, Contribution à l'étude des Algues du Kameroun. Ann. de Biologie lacustre, i, 1906.
30. HARIOT ('89): P. Hariot, Notes sur le genre *Trentepohlia*, Martius. Journ. de Botanique, iii, 1889, p. 345 et seq. (also loc. cit., iv, 1890, p. 85 et seq.).
31. ——— ('91): P. Hariot, Quelques Algues du Brésil et du Congo. Notarisia, vi, 1891, p. 1217 et seq.
32. ——— ('92): P. Hariot, A propos des *Trentepohlia* des Indes Néerlandaises. Journ. de Botanique, vi, 1892, pp. 114–16.
33. ——— ('95): P. Hariot, Liste des Algues recueillies au Congo par M. H. Lecomte. Journ. de Botan., ix, 1895, pp. 242–4.
34. HENRIQUES ('86): J. Henriques, Contribuições para o estudo da Flora d'Africa. Bol. Soc. Broter., iv, 1886 (1887), p. 217 et seq.
35. HEYDRICH ('94): F. Heydrich, Beiträge zur Kenntniss der Algenflora von Ost-Asien, besonders der Insel Formosa, Molukken- und Liu-Kiu-Inseln. Hedwigia, xxxiii, 1894, p. 267 et seq.
36. HIERONYMUS ('95): G. Hieronymus, Algen, in Engler, Die Pflanzenwelt Ost-Afrikas und der Nachbargebiete, Theil c, Berlin, 1895.
37. JADIN ('93): F. Jadin, Algues des îles Mascareignes récoltées en 1890 (Nostocacées). Bull. Soc. Bot. de France, t. xl, 1893, pp. cxlviii–clxxiii.
38. JOSHUA ('86): W. Joshua, Burmese Desmidieae, with Descriptions of new Species occurring in the neighbourhood of Rangoon. Journ. Linn. Soc. Bot., vol. xxi, 1886, p. 634 et seq.
39. KARSTEN ('91): G. Karsten, Untersuchungen über die Familie der Chroolepideen. *Ann.* Jard. Bot. Buitenzorg, x, 1891, p. 1 et seq.
40. KOORDERS ('01): S. H. Koorders, Notiz über die dysphotische Flora eines Süsswassersees *in* Java. Naturk. Tijdschr. Ned. Indie, lxi, 1901, No. 3.
41. LAGERHEIM ('85): G. Lagerheim, Bidrag till Amerikas Desmidié-flora. Öfvers. af K. Sv. Vet.-Ak. Förhandl., 1885, No. 7, p. 225 et seq.
42. ——— ('87): G. Lagerheim, Algologiska Bidrag. II.: Ueber einige Algen aus Cuba, Jamaica und Puerto-Rico. Bot. Notiser, 1887, pp. 193–9.
43. ——— ('88): G. Lagerheim, Ueber Desmidiaceen aus Bengalen nebst Bemerkungen über die geographische Verbreitung der Desmidiaceen in Asien. Bih. till K. Sv. Vet.-Ak. Handl., xiii, Afd. III, No. 9, 1888.
44. ——— ('90): G. Lagerheim, Contribuciones á la Flora algológica del Ecuador. Añales de la Universidad de Quito, iv, 1890, 16 pp. (separate copy).
45. ——— ('92): Ueber einige neue Arten der Gattung *Phyllosiphon*, Kühn. Nuovo Notarisia, Ser. iii, 1892, pp. 120–4.
46. ——— ('93): Chlorophyceen aus Abessinien und Kordofan. Nuovo Notarisia, Ser. iv, 1893, p. 153 et seq.
47. LEMMERMANN ('99): E. Lemmermann, Planktonalgen, in H. Schauinsland, Ergebnisse einer Reise nach dem Pacific (1896–97). Abhandl. Nat. Ver. Bremen, Bd. xvi, 1900, p. 313 et seq.
48. ——— ('05): E. Lemmermann, Die Algenflora der Sandwich-Inseln, in Ergebnisse einer Reise nach dem Pacific, H. Schauinsland 1896–97. Engler's Bot. Jahrb. xxxiv, 1905, p. 607 et seq.
49. ——— ('06): E. Lemmermann, Ueber die von Herrn Dr. Walter Volz auf seiner Weltreise gesammelten Süsswasseralgen. Abhandl. Nat. Ver. Bremen, Bd. xviii, 1906, p. 143 et seq.
50. MAGNUS und WILLE ('82): P. Magnus and N. Wille, Untersuchung der auf der Süsswasserschlange *Herpeton tentaculatum*, Lacep. aus Bangkok in Siam wachsenden Algen (*Cladophora ophiophila*, M. et W.). Sitz.-ber. Ges. Nat. Freunde, Berlin, 1882 (not seen).
51. MARTELLI ('86): U. Martelli, Florula bogosensis: Enumerazione delle piante dei Bogos raccolte dal Dott. O. Beccari nell'anno 1870 con descrizione delle specie nuove o poco note. Firenze 1886 (Algae by Grunow, p. 150 et seq.).

52. MARTENS ('66*): G. v. Martens, Die Tange, in Die Preussische Expedition nach Ost-Asien, Botan. Theil, Berlin, 1866, p. 19 et. seq., and 111, 112.
53. ——— ('70): G. v. Martens, Conspectus Algarum Brasiliae hactenus detectarum. Vid. Meddelels. Nat. For. i Kjøbenhavn, 1870, pp. 297–314.
54. ——— ('71): G. v. Martens, Algae Brasilienses circa Rio de Janeiro a clar. A. Glaziou, horti publici directore, botanico indefesso, annis 1869 et 1870 collectae. Vid. Meddelels. Nat. For. i Kjøbenhavn, 1871, p. 144 et seq.
55. MÖBIUS ('88): M. Möbius, Ueber einige in Portorico gesammelte Süsswasser- und Luftalgen. Hedwigia, xxvii, 1888, p. 221 et seq.
56. ——— ('89): M. Möbius, Bearbeitung der von H. Schenck in Brasilien gesammelten Algen. Hedwigia, xxvii, 1889, p. 309 et seq.
57. —— — ('90): M. Möbius, Algae Brasilienses a cl. Dr. Glaziou collectae. Notarisia, v, 1890, p. 1065 et seq.
58. ——— ('92): M. Möbius, Ueber einige brasilianische Algen. Ber. deutsch. Bot. Ges., vol. x, 1892, pp. 17–26.
59. ——— ('93): M. Möbius, Beitrag zur Kenntnis der Algenflora Javas. Ber. deutsch. Bot. Ges., vol. xi, 1893, p. 118 et seq.
60. ——— ('95): M. Möbius, Ueber einige brasilianische Algen. Hedwigia, xxxiv, 1895, p. 173 et seq.
61. MONTAGNE ('39): C. Montagne, Cryptogamae brasilienses, etc. Ann. Sci. Nat., Bot., Sér. II, vol. xii, 1839, p. 45.
62. NORDSTEDT ('69): C. F. O. Nordstedt, Desmidiaceae, in E. Warming, Symbolae ad floram Brasiliae centralis cognoscendam. Vid. Meddelels. Nat. For. i Kjøbenhavn, 1869, Nos. 14, 15, pp. 195–234.
63. ——— ('77): C. F. O. Nordstedt, Nonnullae algae aquae dulcis brasilienses. Öfvers. Sv. Vet.-Ak. Förhandl., xxxiv, 1877, No. 3, p. 15 et seq.
64. ——— ('78): C. F. O. Nordstedt, De Algis aquae dulcis et de Characeis ex insulis Sandvicensibus a Sv. Berggren 1875 reportatis. Minnesskrift., Lund, 1878.
65. ——— ('80): C. F. O. Nordstedt, De Algis et Characeis. I.: De Algis nonnullis, praecipue Desmidieis, inter Utricularias Musei Lugduno-Batavi. Act. Univ. Lund, xvi, 1879–80, Pt. II, No. iv, pp. 1–20.
66. ——— ('89): C. F. O. Nordstedt, De Algis et Characeis. III. De duabus novis speciebus Desmidiearum e Brasilia. Act. Univ. Lund, xxv, 1889.
67. ——— ('97): C. F. O. Nordstedt, Sötvattensalger från Kamerun. Botan. Notiser, 1897, p. 131 et seq.
68. PRAIN ('05*): D. Prain, The Vegetation of the Districts of Hughli-Howrah and the 24 Pergunnahs. Rec. Bot. Survey, India, iii, No. 2, 1905, p. 328 et seq.
69. RABENHORST ('55*): Beitrag zur Kryptogamenflora Süd-Afrikas: Pilze und Algen. Allgem. Deutsch. Naturhist. Zeit., i, 1855, pp. 280–3.
70. RACIBORSKI ('95): M. Raciborski, Die Desmidieenflora des Tapakoomasees. Flora, Bd. lxxxi, 1895, pp. 30–5.
71. ——— ('00): M. Raciborski, Parasitische Algen und Pilze Javas. I, II, and III. Publ. by Bot. Inst. Buitenzorg, Batavia, 1900.
72. SCHMIDLE ('95): W. Schmidle, Einige Algen aus Sumatra. Hedwigia, xxxiv, 1895, p. 293 et seq.
73. ——— ('97 A): W. Schmidle, Algologische Notizen. II.: *Conferva Sandvichensis*, Ag. Kneucker's Allgem. Bot. Zeitschr., iii, 1897, pp. 3, 4.
74. ——— ('97 B): W. Schmidle, Algologische Notizen. III.: Einige neue und seltene Algen aus Polynesien. Kneucker's Allgem. Bot. Zeitschr., iii, 1897, p. 57 et seq.
75. ——— ('97 C): W. Schmidle, Vier neue von Professor Lagerheim in Ecuador gesammelte Baumalgen. Ber. deutsch. Bot. Ges., Bd. xv, 1897, p. 456 et seq.
76. ——— ('97 D): W. Schmidle, Epiphylle Algen nebst einer *Pithophora* und *Dasya* aus Neu-Guinea. Flora, lxxxiii, 1897, p. 304 et seq.
77. ——— ('97 E): W. Schmidle, Süsswasseralgen, in F. Reinecke, Die Flora der Samoa-Inseln. Engler's Bot. Jahrb., xxiii, 1897, p. 253 et seq.
78. ——— ('97 F): W. Schmidle, Einige Baumalgen aus Samoa. Hedwigia, xxxvi, 1897, p. 277 et seq.

79. SCHMIDLE ('98 A): W. Schmidle, Die von Professor Dr. Volkens und Dr. Stuhlmann in Ost-Afrika gesammelten Desmidiaceen. Engler's Bot. Jahrb., Bd. xxvi, 1898, p. 1 et seq.
80. ——— ('98 B): W. Schmidle, Ueber einige von Prof. Lagerheim in Ecuador und Jamaika gesammelte Blattalgen. Hedwigia, xxxvii, 1898, p. 61 et seq.
81. ——— ('99): W. Schmidle, Algologische Notizen. VIII.: *Batrachospermum Bohneri*, Schmidle n. sp.; IX.: *Phylloplax candelabrum*, Schmidle n. gen. Kneucker's Allgem. Bot. Zeitschr., v, 1899, pp. 2–4.
82. ——— ('00 A): W. Schmidle, Ueber einige von Professor Hansgirg in Ostindien gesammelte Süsswasseralgen. Hedwigia, xxxix, 1900, p. 160 et seq.
83. ——— ('00 B): W. Schmidle, Einige neue von Prof. Dr. Hansgirg in Vorderindien gesammelte Süsswasseralgen. Kneucker's Allgem. Bot. Zeitschr., vi, 1900, p. 17 et seq.
84. ——— ('00 C): W. Schmidle, Drei interessante tropische Algen. Bot. Centralbl., lxxxi, 1900, pp. 417, 418.
85. ——— ('00 D): W. Schmidle, Süsswasseralgen, in Die Flora der deutschen Schutzgebiete in der Südsee (by K. Lauterbach and K. Schumann). Leipzig, 1901, p. 1 et seq.
86. ——— ('01 A): W. Schmidle, Algen aus Brasilien. Hedwigia, xl, 1901, p. 45 et seq.
87. ——— ('01 B): W. Schmidle, Einige Algen, welche Prof. Dr. Volkens auf den Carolinen gesammelt hat. Hedwigia, xl, 1901, p. 343 et seq.
88. ——— ('02 A): W. Schmidle, Beiträge zur Algenflora Afrikas. Engler's Bot. Jahrb., Bd. xxx, 1902, pp. 58–68.
89. ——— ('02 B): W. Schmidle, Schizophyceae, Conjugatae, Chlorophyceae, in A. Engler, Berichte über die botanischen Ergebnisse der Nyassa-See- und Kinga-Gebirgs-Expedition. Engler's Bot. Jahrb., Bd. xxx, 1902, p. 240 et seq.
90. ——— ('03 A): W. Schmidle, Algen, insbesondere solche des Planktons aus dem Nyassa-See und seiner Umgebung. Engler's Bot. Jahrb., Bd. xxxii, 1903, p. 54 et seq.
91. ——— ('04 A): W. Schmidle, Das Chloro- und Cyanophyceenplankton des Nyassa- und einiger anderer inner-afrikanischer Seen. Engler's Bot. Jahrb., Bd. xxxiii, 1904, p. 1 et seq.
92. ——— ('04 B): W. Schmidle, Einige neue Algen aus Java und den Philippinen (gesammelt von A. Usteri, Zürich). Hedwigia, xliii, 1904, pp. 414, 415.
93. TURNER ('92*): W. B. Turner, Algae aquae dulcis Indiae orientalis. K. Sv. Vet.-Ak. Handl., Bd. xxv, No. 5, 1892.
94. WALLICH ('60): G. C. Wallich, Descriptions of Desmidiaceae from Lower Bengal. Ann. Mag. Nat. Hist., Ser. 3, v, 1860, p. 184 et seq.
95. WARD ('84): H. Marshall Ward, Structure, Development, and Life-history of a Tropical Epiphyllous Lichen. Trans. Linn. Soc., Ser. II, vol. ii, 1884.
96. WEST ('02): G. S. West, On some Algae from Hot Springs. Journ. of Bot., xl, 1902, p. 241 et seq.
97. —— ('04): G. S. West, West Indian Freshwater Algae. Journ. of Bot., xlii, 1904, p. 281 et seq.
98. WEST and WEST ('93): W. and G. S. West, Algae from the West Indies. Journ. Linn. Soc. Bot., xxx, 1893–5, p. 264 et seq.
99. ——— ('95): W. and G. S. West, A Contribution to our Knowledge of the Freshwater Algae of Madagascar. Trans. Linn. Soc. Bot., Ser. 2, v, 1895, p. 41 et seq.
100. ——— ('96): W. and G. S. West, Algae from Central Africa. Journ. of Bot., vol. xxxiv, 1896, p. 377 et seq.
101. ——— ('97 A): W. and G. S. West, Welwitsch's African Freshwater Algae. Journ. of Bot., vol. xxxv, 1897, p. 1 et seq.
102. ——— ('97 B): W. and G. S. West, Desmids from Singapore. Journ. Linn. Soc. Bot., xxxiii, 1897, p. 156 et seq.
103. ——— ('99): W. and G. S. West, A Further Contribution to the Freshwater Algae of the West Indies. Journ. Linn. Soc. Bot., xxxiv, 1899, p. 279 et seq.
104. ——— ('02 A): W. and G. S. West, Freshwater Chlorophyceae, in J. Schmidt, Flora of Koh Chang. Bot. Tidsskrift, xxiv, 1902, p. 157 et seq.
105. ——— ('02 B): W. and G. S. West, A Contribution to the Freshwater Algae of Ceylon. Trans. Linn. Soc. Bot., Ser. 2, vi, 1902, p. 123 et seq.

106. WILLE ('84): N. Wille, Bidrag till Sydamerikas Algflora. Bih. K. Sv. Vet.-Ak. Handl., viii, No. 18, 1884.

107. ——— ('03): N. Wille, Ueber einige von J. Meynhardt in Südafrika gesammelte Süsswasseralgen. Österr. Bot. Zeitschr., 1903, p. 89 et seq.

108. WITTROCK ('78): V. B. Wittrock, Oedogonieae Americanae, hucusque cognitae. Bot. Notiser, 1878, pp. 133–45.

109. ZANARDINI ('72): J. Zanardini, Phycearum indicarum pugillus a cl. Eduardo Beccari ad Borneum, Singapoore et Ceylanum annis 1865–7 collectarum. Mem. d. Reale Istit. Veneto, xvii, 1872, p. 129 et seq.

110. ZELLER ('73ª): G. Zeller, Algae collected by Mr. S. Kurz in Arracan and British Burma. Journ. Asiat. Soc. Bengal, xlii, 1873, pp. 175–93; also Hedwigia, 1873, p. 168 et seq.

111. ——— ('76): G. Zeller, Algae Brasilienses circa Rio de Janeiro a Dr. A. Glaziou, horti publici directore, collectae. Vid. Meddelels. Nat. Foren. i Kjøbenhavn, 1876, p. 426 et seq.

A Note on Wounded Calamites.

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With Plate XXIII and four Diagrams in the Text.

AN interesting case of the formation of callus wood in a wounded Calamite is shortly described and figured by Seward[1] in his 'Fossil Plants'. The stem, from which several sections were cut, is about 3 mm. in diameter, and is, so far as I am aware, the only case in which such a formation has been noted.

Two specimens showing the healing of wounds in very much larger stems have recently come under my observation, and as in both cases the wound was deeper than in the Cambridge stem they show more complex arrangements in the healing tissues.

One of these specimens is represented by a series of three slides in the collection of the Manchester University Museum, Nos. R 100, R 101, and R 102, and is entered in the catalogue as a 'branching stem.' The woody cylinder, which alone is preserved, is 35 mm. in diameter, and shows, therefore, a considerable development of secondary tissue. This is seen in general view in Diagrams 1, 2, and 3. The other specimen is a stem of about 25 mm. in diameter, also without cortex, of which the two slides are in my possession. I obtained them from Mr. Lomax, labelled as coming through a node, but comparison with the first series makes it clear that they are also through a wound of a very similar nature (see Diagram 4).

In both these cases the wound was so deep as to pass right through the tissues to the pith, thus breaking through the vascular cylinder. As a result, the formation of new tissue curved round the open ends of the broken ring and formed a quantity of wood in the pith cavity in inverse orientation to the normal strands. In Professor Seward's specimen, which he kindly lent me for comparison with mine, the wound did not lie so deeply, but stopped short of the primary cylinder, leaving the bundle

[1] A. C. Seward, Fossil Plants, vol. i, 1898, pp. 319, 320, Text-fig. 80.

canals undisturbed, as can be seen in his diagram (*loc. cit.*, Fig. 80). The callus wood in his case, therefore, though bending across to close up the wound, is simply formed in the normal direction. As the cylinder was not broken through, the edges could not inroll, thus making the case much simpler than in the larger stems with deeper wounds.

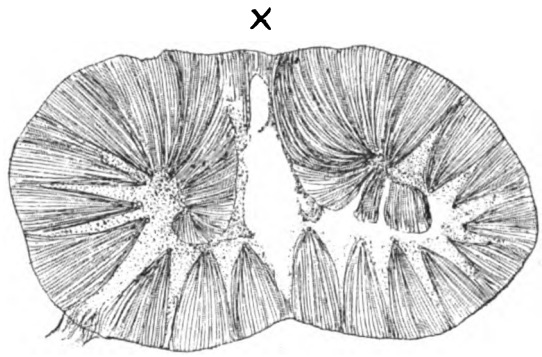


DIAGRAM 1. Trans. sect. of wounded Calamite, × 2. Wound at *x*, externally just closed up. In the pith cavity on either side are the curved wedges of callus wood completely inrolled. R. 100, Manchester University Coll.

In the latter, as may be seen in Pl. XXIII, Fig. 1, *c*, the wound-cambium was very active in the neighbourhood of the injury, curving in and out round the primary bundles, adding new tissue where there was room for it. The injured primary strands, with small quantities of secondary tissue associated with them, have been cut off and isolated from the rest of the wood by this cambium band (see Pl. XXIII, Fig. 1, *p*), and had evidently partly decayed while the rest of the stem was still vigorous, judging from the different states of preservation of the two tissues.

That there was a definite formation of inverted wood in addition to the callus wood curving round the edges may be seen by the position of the cambium zone, which extends round the primary strands some distance beyond the actual wound-gap. This cambium does not simply form a mass of new tissue, but tends to produce bundles facing the normal ones (see Pl. XXIII, Figs. 1 and 2, *i*). The inverted masses of tissue are thinner-walled than the bulk of the woody elements, but are thickened and definitely pitted, as examination with the high power clearly reveals. Where the callus wood is cut obliquely it shows that some of the tracheides are very irregular and much curved. This has been described

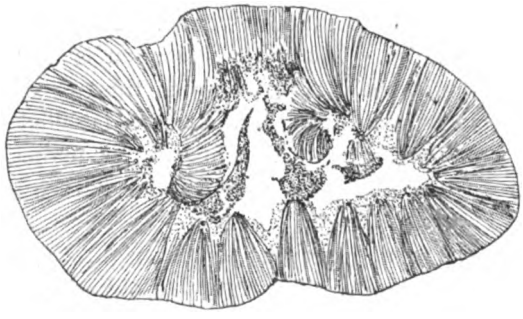


DIAGRAM 2. Neighbouring sect. R. 101. × 2. Externally wound further closed, inrolled wedges of callus less developed.

as occurring commonly just at the point of exit of branches in normal Calamite stems (see Williamson [1], Fig. 28, Pl. XXI, and Fig. 17, Pl. XX), and is also a character of the callus wood of many plants, see Küster, p. 177 [2].

The exact shape of the wound is not to be determined from the small number of sections available, and may have extended for some distance down the stem, where it may not have closed completely; in those sections which we have, however, the wound was externally closed up, and the wood formed outside it (as in Diagram 3) is entirely normal in appearance. As the figures show, the total thickness of the normal secondary wood is greater on the injured than on the uninjured side.

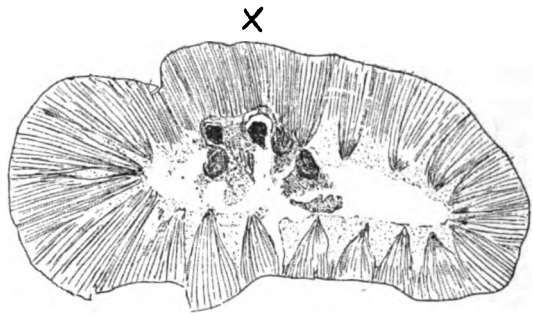


DIAGRAM 3. Neighbouring sect. R. 102. × 2. No inrolled wedges, groups of wood associated with primary bundles cut off by cambium, and inverted strands facing them in pith cavity. Cf. Fig. 1, Pl. XXIII.

The formation of inverted strands of wood in the pith is to be looked upon simply as a result of the stimulus of the wound, and the physical conditions and space opened out by the breaking of the vascular cylinder. It is hardly to be supposed that they are of phylogenetic importance, or in any way to be compared with the inverted strands in the Pteridosperms and Gymnosperms. They probably find a close analogy among the fossils in the inrolled secondary wood in a *Lepidodendron* described and figured by Williamson [3], p. 292, and Fig. 20, Pl. XLIII, in which the dichotomizing cylinder did not close up to form two complete rings as is usual,

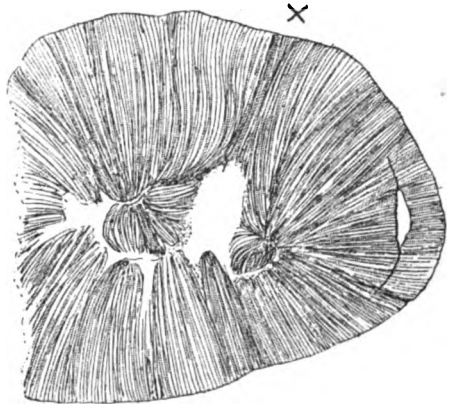


DIAGRAM 4. Trans. sect. of second specimen. × 2. Wound at *x*, externally closed, but showing in pith inrolled strands of wood.

[1] W. C. Williamson, On the Organization of Fossil Plants, Pt. IX, Phil. Trans., 1878.

[2] E. Küster, Pathologische Pflanzenanatomie, 1903.

[3] W. C. Williamson, On the Organization of the Fossil Plants of the Coal-Measures, III, Phil. Trans., 1872.

but each half remained open, and the secondary tissue inrolled in the pith and 'endeavoured to accomplish' the closure of the cylinders. Among recent plants, wounds and pathological conditions frequently lead to the formation of unusually orientated and abnormal wood [1].

Such specimens as these wounded Calamites, reveal in a particularly striking fashion that we have in the fossil tissues the remains of plants which were once alive, combating with similar accidents in their environment to those which assail the plants of to-day, and were stricken down and fossilized in the midst of their activities. This, as well as the great rarity of such structures in Calamites, must justify the present short note.

DESCRIPTION OF PLATE XXIII.

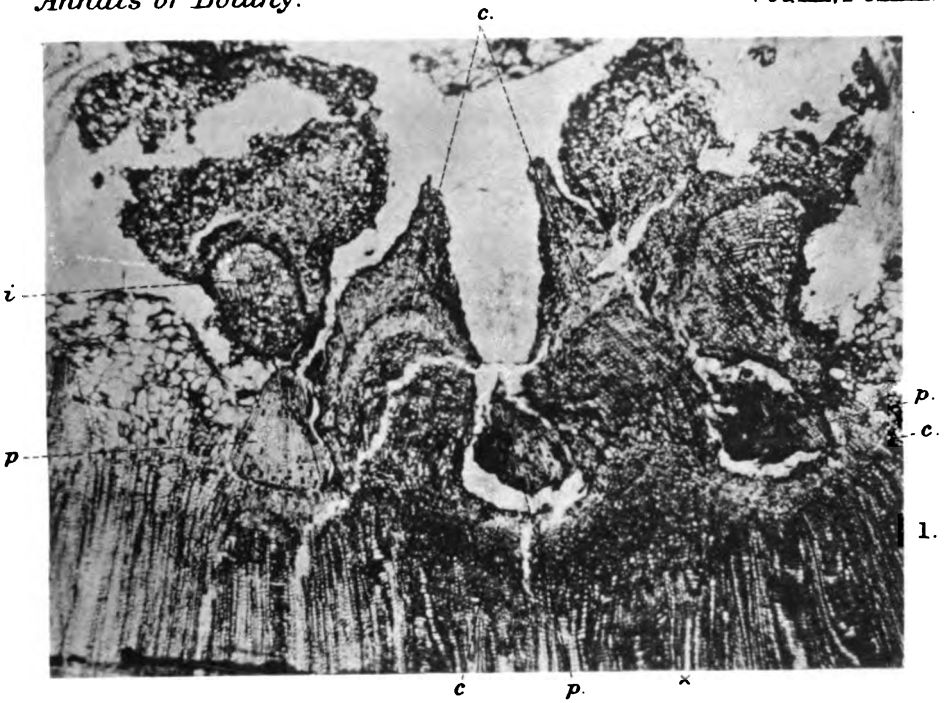
Illustrating Miss Stopes's Paper on wounded Calamites.

Photos. by Mr. James Lomax.

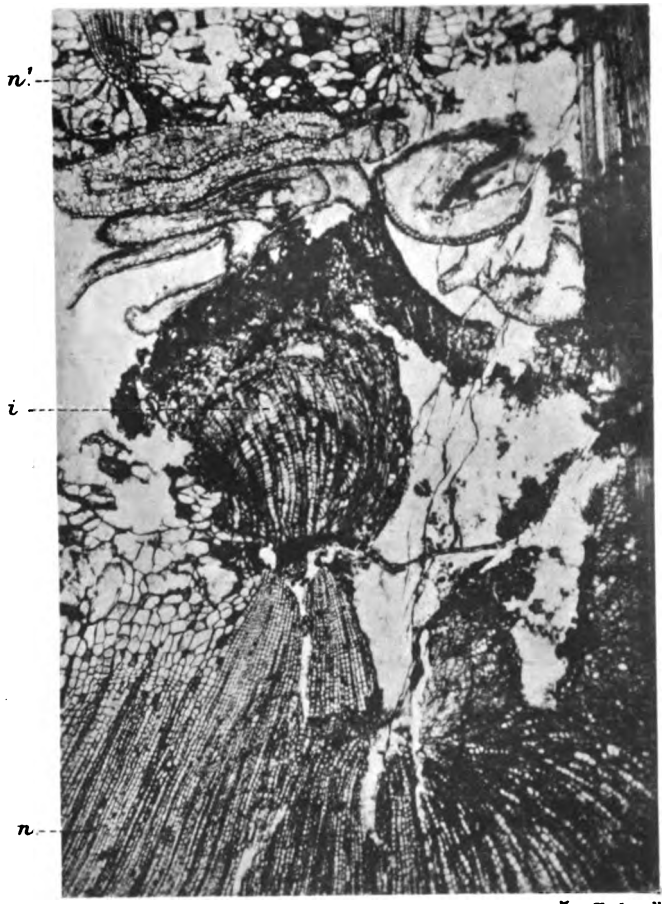
Fig. 1. Part of section shown in Diagram 3, enlarged. × 12. *p*, groups of primary bundles and associated secondary tissue cut off and isolated by (*c*) cambium, which has produced (*a*) additional tissue between the primary strands, and also (*i*) inverted strands opposite them. Note the very wavy course of the cambium resulting from this.

Fig. 2. Part of section shown in Diagram 2, enlarged. × 18. *n*, normal wood. *n'*, normal bundles at other side of stem. *x*, position of wound. *i*, inverted strands opposite primary bundles.

[1] P. Sorauer, Handb. d. Pflanzenkrankheiten, Pls. II, IV, &c.



1.



2.

Lamax, phot. Huth. coll.

STOPES — WOUNDED CALAMITES.

The Gametophytes and Embryo of the Cupressineae with special reference to Libocedrus decurrens.

BY

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With Plates XXIV–XXVI.

INTRODUCTION.

THE series of studies on the Gymnosperms which the writer has published during the last three years, and of which the present memoir is the fourth, was undertaken with the idea that a detailed knowledge of the vestigial structures associated with the gametophyte generation might throw some light on the phylogenetic relationships of the various sub-groups of the Coniferales, and that the data, accumulated from the facts concerning such structures, might eventually be of some service in contributing to our knowledge as to the origin of the Gymnosperms in general. Although the Coniferales are second only to the Angiosperms as a dominating race of seed-plants, they are, nevertheless, a very ancient group, extending, as the fossil evidence would seem to indicate, as far back as the carboniferous period. In attempting to construct a phylogeny of a group of plants of such great antiquity from a study of recent forms, one must naturally rely almost entirely upon a study of those structures, the nature of which will throw light upon ancestral affinities. For instance, the prothallial cells in the microspore, the structures in the pollen-tube, the organization and form of the sperm cells, the megaspores, the tapetum, the megaspore membrane, the formation of the female prothallium, the structure of the archegonia, the ventral canal cell, the process of fertilization, and the development of the embryo, all show phases of phylogenetic interest. With the exception of the early history of the male gametophyte, all of these more or less vestigial structures are buried within the tissues of the sporophyte, and therefore are less liable to be modified and specialized by external factors, and more likely to preserve characters which are obviously primitive than those of the sporophyte. That the sporophyte contributes evidence of great value is clearly indicated by Professor Jeffrey's

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('03, '05) investigations on the anatomy of the vascular system. Indeed, in any scheme which may be advanced concerning the phylogeny of this ancient group, the sporophyte as well as the gametophytes must constitute an essential part, for it is mainly from the point of view of anatomy that we can hope to correlate the evidence from the fossils—the structures associated with the sexual generation being so much more perishable.

In this series of studies no attempt has been made to follow any natural sequence in the types selected. It was thought that good results might be obtained by working up the available material first; leaving the more inaccessible and perhaps more interesting types, such as *Arancaria* and *Agathis*, for future investigations. The present paper is merely intended as an addition to the knowledge which we have already received from the works of Strasburger ('79), Arnoldi ('01), Land ('02), Coker ('03), and the present writer ('04) on the gametophytes of the Cupressineae. Special attention has been given to *Libocedrus*, because, up to the present, no observations have been recorded concerning the sexual generation of this genus. The work was commenced in the Laboratory of Stanford University, California, but most of it was carried on in the Jodrell Laboratory, Kew Gardens. To Lieut.-Col. Prain, C.I.E., F.R.S., Director of the Royal Gardens, Kew, and to Dr. D. H. Scott I wish to express my sincere thanks for extending to me the privileges of the Laboratory. My thanks are also due to Mr. L. A. Boodle, F.L.S., whose constant attention and many kindnesses aided materially towards the completion of the work.

Material and Methods.

The collection of material for this investigation was commenced in March, 1903, and was continued at short intervals as time permitted. The collections extended over a period of two years. All of the material was obtained from trees growing in the arboretum of Stanford University. The Cupressineae are here richly represented in both genera and species, and the climatic conditions seem very favourable for the development and maturing of the fruit. Every year there is an abundance of cones on most of the trees.

The methods used are the same as those which I adopted in previous investigations on the Conifers (Lawson '04), and gave satisfactory results.

The Male Gametophyte.

In *Libocedrus decurrens* pollination takes place during the last week in March and early in April; the period extending from two to three weeks. The first collections of the microsporangia were taken early in March, and at this time the pollen seemed fully developed—each grain having a thick exine and containing two nuclei, the latter being separated from each other by a distinct membrane. In the division of the spore before its

discharge from the microsporangium, *Libocedrus* agrees with the majority of other Cupressineae, but apparently differs from *Juniperus* and *Cupressus*, where, according to Strasburger ('92) and Coker ('04), there is no division of the microspore nucleus until some time after pollination.

During the period of pollination the micropyle exudes a small globule of transparent liquid. The purpose of this is no doubt to catch the pollen more effectively, for an examination of the globule upon a slide under the microscope showed it to contain several pollen-grains. The same thing has been observed in other Conifers—especially in *Taxus baccata*, where the globule of liquid is particularly obvious. It was observed more frequently during the early morning, before the heat of the day had time to cause evaporation.

The pollen-grain of *Libocedrus* is—like that of other Cupressineae—very small. It contains but two cells, and in this regard resembles all other Conifers except the Abietineae and Podocarpeae. No trace of the survival of sterile prothallial cells was found. The single or first division which takes place, and which results in the organization of the generative and tube-nuclei, is the only division in the male gametophyte preceding that resulting in the formation of the body and stalk-nuclei. In view of more recent studies on the pollen, Coker's statement, that 'In the absence of any sterile prothallial cells, *Taxodium* agrees with the Cupressineae and *Taxus*, and differs from all other Conifers and Cycads,' is misleading; for we now know that these prothallial cells are not of general occurrence among the Coniferales, but are probably confined to the Abietineae and Podocarpeae At the time of the shedding of the pollen in *Libocedrus*, the tube-nucleus is centrally situated, and is considerably larger than the generative nucleus. The latter was invariably found lying to one side near the spore-wall.

Several spores find their way to the apex of the nucellus, and here retain their positions for three or four weeks without any further visible changes. Meantime the cells lining the upper part of the integument grow inwards, and gradually close the micropyle in essentially the same manner as they do in *Sequoia* and *Cryptomeria* (Lawson, '04). The next indication of the further germination of the spore was its enlargement to about twice or three times its original size, and the appearance of the pollen-tube. The latter at first pushes out over the top of the nucellus, but very soon begins its downward penetration into the nucellar tissue, without any indication whatever of branching. Very young pollen-tubes were observed early in May, which was just about a month after pollination. There are usually three or four pollen-tubes present, but as many as six have been observed penetrating the tissue of a single nucellus. The courses they follow diverge very little out of the straight lines between the original points of penetration and the archegonial chamber below. Their growth is accompanied by the usual breaking down and disorganization of the nucellar tissue through

which they pass. All of the tubes penetrate the tissue near the apex of the nucellus. In no case was I able to find any of the tubes growing down between the nucellus and the integument, as so commonly occurs in *Sequoia sempervirens* (Lawson, '04).

Just previous to the penetration of the pollen-tube into the nucellar tissue, the generative nucleus enlarges and prepares for division. That this division occurs when the pollen-tube is very young was shown by the fact that in material collected during the second week in May, when the tube had penetrated but a very short distance into the nucellus, the body-cell and stalk-nucleus were completely organized. The body-nucleus was easily distinguished from the stalk-nucleus because of its greater size, and also because it very soon becomes surrounded by a cell membrane. The young tube at this time thus contains the large body-cell and the stalk- and tube-nuclei—the latter lying freely in the tube-cytoplasm. The body-cell is at first distinctly oval in form, and its long axis lies parallel to the long axis of the tube. Its nucleus is quite three or four times the size of the tube or stalk-nuclei. The latter structures are almost equal in size, and it is only from their position that they may be distinguished from one another; they are, however, always found in advance of the body-cell. The appearance of the body-cell, from material killed May 31, is shown in Pl. XXIV, Fig. 6. It will be seen that the cell is not so much oval in form as in earlier stages, and that its cytoplasm is very granular—containing more starch and other food substances than the tube-cytoplasm.

By June 1 the tube has completely penetrated the nucellus. Meantime the female prothallium has been developing rapidly, and the archegonia are fully organized and nearly ready for fertilization by the time the tip of the tube reaches the archegonial chamber. During its entire growth the contents of the tube are always found at the tip. Just about the time the archegonial chamber has been reached the body-cell divides, and as a result of this division two distinct male cells of equal size are formed. Fig. 6 represents the body-cell preparing for division. Fig. 7 represents the two male cells very soon after they have been formed. Fig. 6 was drawn from material killed May 31, and Fig. 7 June 1. As may be seen from Fig. 7, the two male cells are not quite spherical in form, but each of them becomes spherical as soon as they separate from each other and before the archegonia have been reached. It will also be observed that the nucleus of each male cell is very large, with a single conspicuous nucleolus, and the chromatin, in the form of small, uniform granules, suspended on a network of linin.

The organization of two male cells of equal size seems to be a constant character of the Cupressineae (and I consider *Taxodium* and *Cryptomeria* as members of this group). The only exception which has so far been recorded is that of *Cupressus Goweniana*, in which Juel ('04) describes the

body-cell as giving rise to a complex of cells. Realizing the importance of such a condition, I have made a careful study of the pollen-tube in two other species of *Cupressus*, and the conditions found there were almost identical with those described above for *Libocedrus*. In both species studied I found that two male cells of equal size were organized, and that the stalk- and tube-nuclei in addition to these constituted the entire nuclear structures of the pollen-tube. The two male cells were also followed through the fertilization stages. I am therefore compelled to regard the condition described by Juel in *Cupressus Goweniana* as simply an interesting abnormality. In addition to the two species of *Cupressus* which I have examined, the normal condition of two male cells of equal size occurs in *Taxodium*, *Cryptomeria*, *Juniperus*, *Thuja*, and *Chamaecyparis*. The only other Conifer outside of the Cupressineae in which two distinct equal male cells have been observed is *Sequoia* (Shaw, '96; Lawson, '04). In all other Conifers of which we have record the male cells are either unequal in size (*Taxus*, Belajeff, '93; *Podocarpus*, Coker, '02; *Torreya taxifolia*, Coulter and Land, '05), or they are represented only by nuclei as in the Abietineae (Dixon ('94), Blackman ('98), Coulter and Chamberlain ('01), Ferguson ('01)) and in *Cephalotaxus* (Lawson, '07).

As shown in Fig. 7, the male cells become packed with starch grains, and in this respect resemble other members of the Cupressineae. As soon as the male cells are formed the contents of the pollen-tube are discharged into the archegonial chamber. The pollen-tube itself apparently does not carry the male cells into the archegonium, as it does in the Abietineae and some other Conifers, but its function evidently ceases when the archegonial chamber has been reached and its contents discharged. As I shall point out later, the archegonia throughout the Cupressineae are arranged in a single group with a common layer of jacket-cells, and with their necks opening into a common cavity or chamber. It is into this common chamber that the contents of the various pollen-tubes are discharged. As the archegonia are numerous, their arrangement in a single group makes it possible and easy for each male cell to function, so that the contents of each pollen-tube may accomplish the fertilization of two separate archegonia. These conditions seem to offer a fair explanation why one of the male cells is not dwarfed and functionless as it is in many other Coniferales.

The history of the male gametophyte of *Libocedrus* resembles that of *Thuja* probably more closely than any other of the Cupressineae. It agrees in all essential points with the account given by Land ('02) of *Thuja occidentalis*, and also with my own observations on *Thuja orientalis*. The mature pollen of the latter species has but two nuclei—the generative and tube. The pollen-tubes develop and penetrate the nucellar tissue in identically the same fashion as they do in *Libocedrus*. Pl. XXIV, Fig. 1 shows a young pollen-tube, and Fig. 2 shows at least four of them at a later stage.

Fig. 3 represents a young pollen-tube more highly magnified, indicating the appearance and position of the body-cell and tube-nucleus. As shown in Fig. 4, the older body-cell of *Thuja orientalis* differs slightly from that of *Libocedrus* in having a greater quantity of starch and food granules in its cytoplasm. Fig. 5 represents a mature male cell just previous to its entrance into an archegonium.

From Land's ('02) account of *Thuja*, Coker's ('04) account of *Taxodium*, and my own observations on *Cryptomeria* ('04), *Libocedrus*, *Thuja*, *Cupressus*, and *Chamaecyparis*, there seems to be a striking uniformity throughout the Cupressineae in regard to the history of the male gametophyte. Such a close similarity in the microspores and pollen-tube structures apparently does not prevail between the members of any other group of the Coniferales.

The Female Gametophyte.

The first collections of material for the study of the megaspores in *Libocedrus* were taken early in March, but it was found that the megaspore mother-cells do not become differentiated until just about the time of pollination. Pl. XXIV, Fig. 8 represents a section of an ovule taken March 23. At this time the integument extends for a considerable distance beyond the apex of the nucellus, and the micropyle is open. The nucellus itself is quite large and flat on the top. The megaspore mother-cells make their appearance in the middle region of the nucellus in a position in line with the point of insertion of the base of the integument, as indicated in Fig. 8. Two mother-cells are generally developed; occasionally but one was found. They very soon become differentiated from the surrounding tissue by their conspicuously large nuclei and their densely granular cytoplasm. Before dividing they become three or four times the size of the neighbouring cells. Fig. 9 represents two mother-cells lying side by side just before the first division. The spindle stages of this division were not found, so that I am unable to give an account of the chromatin at this critical time. Numerous preparations showed the megaspores after division, and from a study of these there is no doubt that each mother-cell dividing twice gives rise to four megaspores. Fig. 10 shows four of the cells after the first division, and Fig. 11 represents a section through eight spores soon after the second division. The spores are arranged in two rows—four in each row—and have the appearance of those found in *Cryptomeria* rather than the arrangement figured by Coker ('04) for *Taxodium*. In *Taxodium*, however, there is but a single mother-cell organized, while in *Cryptomeria* there are three or four. At the stage shown in Fig. 11 all of the megaspores in *Libocedrus* look alike, and it is quite impossible at this time to say which one will develop into the prothallium. Surrounding the spores there is a single layer of loose cells, which may represent a tapetum. If such, it is a very poorly developed one, and by no means resembles that in *Taxodium*

(Coker, '04) or in the Abietineae. All trace of this layer of cells becomes lost soon after the germination of the functional megaspore.

It seems that only one of the megaspores germinates; the others, along with the layer of loose cells, are presumably absorbed by the growing young prothallium. The functional spore at first increases enormously in size, and its growth is accompanied by a rapid and successive free nuclear division. Pl. XXV, Fig. 12 shows the condition of the spore three weeks after the stage represented in Pl. XXIV, Fig. 11. The megaspore as shown in Fig. 12 contains several large vacuoles separated from each other by irregular strands of cytoplasm, and in the cytoplasm are numerous free nuclei in various stages of mitosis. At this stage a very distinct but very thin megaspore membrane was observed. With the further increase in the size of the megaspore or young prothallium the vacuoles within it also increase and eventually flow together. From a study of these early stages in *Libocedrus* and many other Conifers it would seem that the large central vacuole, which is always present, performs a most important function in the growth of the young prothallium. The megaspore being confined within the sporangium, the young prothallium finds itself completely surrounded by nucellar tissue. Therefore, in order to rapidly produce prothallial tissue, it must not only increase greatly in size, but a rapid and plentiful supply of food material is necessary. Both of these objects must be accomplished by a pressure from within. The vacuole always keeps the parietal layer of cytoplasm at the periphery of the spore in close touch with the surrounding cells of the nucellus, and owing to the osmotic pressure which such conditions must necessarily bring about, the food substances from the surrounding tissue may be readily absorbed.

The manner in which the nucellar tissue of the prothallium becomes organized is not unlike that found in many other Conifers. By the first week in May the central vacuole has reached an enormous size, but as yet there is no trace of cellular tissue having been formed. Fig. 13 represents a section of the lower half of the prothallium at this time. Here the thin parietal layer of cytoplasm may be seen lining the spore membrane, and in it numerous free nuclei lie embedded at more or less regular intervals. The great size of the vacuole may be readily seen by comparing it with the size of the nuclei. The parietal layer of cytoplasm is a mere film; as shown in Fig. 14 it is quite as thin as the diameter of the nucleus. Very soon after the stage represented in Fig. 13 the parietal layer increases to twice or three times its original thickness, and meantime free nuclear division has progressed and the nuclei lie much closer together. The last mitosis of the free nuclei results in the formation of cell-walls. These walls, however, only separate the nuclei from one another, the resulting primary cells being exposed on the inner side to the sap of the vacuole in the manner first described by Sokolowa ('90) for other Conifers. By their growth inward these primary

prothallial cells ('alveoli') gradually encroach upon the space occupied by the vacuole, and as nuclear division continues numerous cross-walls are formed. The majority of the nuclei, however, are found on the inner exposed side of the primary cells, and seem to completely surround the diminishing vacuole in the manner shown in Fig. 15. It will be seen that the formation of the prothallial tissue in *Libocedrus* is essentially the same as that described by Coker ('04) for *Taxodium*. In *Taxodium*, however, Coker finds that cell-formation usually begins in the archegonial region earlier than it does in the basal region of the prothallium. This is not the case in *Libocedrus*, for the upper and basal regions develop permanent tissue about the same time. The central region seemed to be the last to develop cell-walls. In several cases traces of the diminishing central vacuole were observed, even after the archegonia had been organized.

In his investigation of the gametophytes of *Thuja occidentalis*, Land ('02) did not study the development of the megaspores or the formation of the endosperm. Coker ('04), however, has given a brief account of the development of the megaspores in *Thuja orientalis*. My own observations of the megaspores of this latter species of *Thuja* do not agree entirely with those of Coker, although they do in the main. Coker finds that the single mother-cell becomes evident among the conspicuous spongy tissue, with its long axis often at right angles to the longitudinal axis of the ovule. This single mother-cell gives rise to four spores, which are arranged in the form of a tetrad. They are never arranged in a straight row, but are packed close together in such a way that only two or three nuclei may be seen in one section. I was unable to confirm these observations from my own preparations. In the first place I find that the tapetum, especially in the mother-cell stage, is not very conspicuous. I also find that there are generally two spore-mother-cells developed. Coker, however, states that occasionally two, or even three, may be differentiated. Many of my preparations show eight megaspores arranged in a single group, and they resemble very closely those which I have figured and described above for *Libocedrus*.

In regard to the development of the prothallium in *Thuja*, as yet no observations have been recorded. I have therefore carefully followed out the formation of the endosperm in *T. orientalis*, and record my observations here along with *Libocedrus* because these two genera seem to be very closely related. Only one megaspore germinates, and this is accompanied by the usual rapid free nuclear division and the subsequent development of the large central vacuole, with a thin parietal layer of cytoplasm at the periphery. The formation of the parietal endosperm tissue takes place in identically the same manner as described above for *Libocedrus*. Fig. 15 represents a section of the lower half of a young prothallium with the primary prothallial cells very much elongated, and by their growth gradually

diminishing the central vacuole. The nuclei, during this growth of the primary cells, take up their characteristic position, on the inner side exposed to the vacuole. The final formation of permanent tissue takes place in the manner described above for *Libocedrus* and *Taxodium* (Coker, '04).

In *Libocedrus*, *Thuja*, *Cupressus*, and *Chamaecyparis* there is a distinct but poorly developed megaspore membrane at the time the archegonia are formed. An accurate account of the distribution and character of this membrane has already been given by Thomson ('05).

During the development of the central cells the sterile tissue at the apex of the prothallium grows forward, leaving the archegonia behind. The result of this forward growth is the formation of a large, deep, cup-like depression, or archegonial chamber, at the base of which the neck-cells of all the archegonia are closely clustered together. It is into this chamber that the contents of the various pollen-tubes are eventually discharged. While the male cells of the Cupressineae present characters which are apparently more primitive than those of the Abietineae, it must be remembered that their function and form are correlated with this highly specialized condition of the grouping of the archegonia. The formation of a common archegonial chamber into which the tube contents are discharged makes it possible for both male cells to function, and these structures are therefore not reduced or dwarfed as is the case with so many other Coniferales. This, however, will be discussed more at length under the head of fertilization.

The Archegonia.

The archegonia in *Libocedrus* are always arranged in a single group, and vary in number from ten to fifteen. The initials become differentiated as a group of cells at the apex of the prothallium as soon as the permanent tissue of the latter has been organized. They are all of superficial origin. The youngest initials observed were several times larger than the neighbouring cells, and their nuclei were very conspicuous and stained deeply. The further growth of the archegonia is evidently very rapid. The next stages observed showed the neck-cells, and these seemed to vary in number from four to six, arranged in a single tier, no periclinal walls being formed.

As soon as the neck-cells have been formed the central cell becomes elongated enormously, and the nucleus, which also increases in size, takes up a position in the cytoplasm very near the neck. Meantime quite a large vacuole makes its appearance in the central region of the archegonium. The central nucleus now undergoes division, and this results in the organization of the egg-nucleus and the ventral canal-nucleus. All of the central nuclei of a single archegonial complex seem to divide nearly simultaneously. One preparation showed the nuclei of five neighbouring central cells in the spindle stage. Other preparations showed these nuclei in various stages of mitosis. All of the Cupressineae so far investigated, including *Taxodium* (Coker, '04)

and *Cryptomeria* (Lawson, '04), fail to develop a membrane between the egg and ventral canal-nuclei. A similar condition is also found in *Sequoia* (Lawson, '04), *Podocarpus* (Coker, '02), *Cephalotaxus* (Lawson, '07), and probably also *Torreya* (Robertson, '04). In fact, it is only in members of the Abietineae that a ventral canal-*cell* has been found. In all other Conifers this primitive vestigial structure seems to be represented only by a nucleus. It has been a matter of considerable difficulty to determine which type of archegonium among the Coniferales is the most primitive, and this has given rise to much speculation. But now that so many forms have been investigated, it seems to me that the type of the Cupressineae, and all others where the ventral canal-cell is represented only by a nucleus, ought to be regarded as less primitive than that where a definite ventral canal-cell is formed, which seems to be characteristic of the Abietineae. This is merely emphasizing a point of phylogenetic importance which I recently suggested in the case of *Cephalotaxus* (Lawson, '07), and which has impressed itself more strongly upon me by a study of *Libocedrus*, *Thuja*, *Cupressus*, *Chamaecyparis*, and *Juniperus*.

In all of these types the size, shape, internal structure, and the method of grouping of the archegonia are very much alike. They all very much resemble *Thuja occidentalis*, first described by Land ('02). There are one or two points, however, in Land's description which I was unable to confirm. He states that almost immediately after the last anticlinal wall is formed the neck-cells begin to disorganize, and they are soon almost entirely assimilated by the central cells, which at this time are increasing in volume at a very rapid rate. My own observations convince me that the neck-cells in all of the Cupressineae which I have examined remain intact until just about the time of fertilization. Fig. 16 represents a section through an archegonial complex at the time the ventral canal-nucleus is being organized. It will be observed that the neck-cells are undisturbed. In regard to the number of archegonia, Land states that in *Thuja occidentalis* the complex usually contains but six. Fig. 18 represents a cross-section of the group of archegonia in *Thuja orientalis* showing the presence of twenty-three. Several preparations showed as many as twenty-four. In none of the Cupressineae which I have examined have I found as few as six archegonia in a single complex. In all other particulars the archegonia of this latter species of *Thuja* agrees with Land's description. The organization of the ventral canal-nucleus takes place in identically the same manner as in the former species. Fig. 19 shows the position of the central nucleus during its division. Fig. 21 represents the spindle of this mitosis more highly magnified. Fig. 20 indicates the relative position of the egg-nucleus and ventral canal-nucleus some time after the organization of the latter. It will be noticed that the egg-nucleus has enlarged considerably.

Enveloping the archegonial complex in *Libocedrus*—and this is true

also for the other Cupressineae which I have investigated—there is a single layer of jacket-cells. Compared with those of other Gymnosperms these cells are poorly developed, being very small, while the walls separating them from the central cell are almost as thin as the walls of the ordinary endosperm-cells. They become differentiated as jacket-cells quite early—before the neck-cells are formed—and soon become filled with a dense cytoplasm, due no doubt to the accumulation of food substances, and as a consequence stain much more deeply than the neighbouring cells. With the growth of the archegonia the jacket-cells divide and increase in number, but their multiplication ceases when the central cells have reached their full size. Many of them at this time are binucleate, a peculiarity which has also been observed in *Taxodium* (Coker, '04). In *Cryptomeria* they may even be multinucleate (Lawson, '04). The general appearance and distribution of the jacket-cells may be seen in Pl. XXV, Figs. 16, 18, and Pl. XXVI, Figs. 25 and 30.

The relationship of these jacket-cells to the nutrition of the egg has been a matter of much discussion for the Gymnosperms in general, especially since Arnoldi ('00) described and figured the actual passage of the jacket-cell nuclei through the cell-walls and into the cytoplasm of the egg, thus giving rise to the structures known as 'proteid-vacuoles.' This subject has, however, been thoroughly investigated quite recently by Stopes and Fujii ('06) in the case of the Cycads, *Ginkgo* and *Pinus*, and also by Chamberlain ('06) in the case of *Dioon*. From the account given by Stopes and Fujii it appears that the thick wall between the jacket-cells and the egg is pitted, but each pit is closed by a thin membrane which is perforated only by 'plasmodesmen.' They therefore point out the impossibility of nuclei, starch grains, or protein grains being transferred bodily from the jacket-cells into the egg. They further suggest that the jacket-cells are glandular or secretory, and render the storage food of the endosperm soluble and available for absorption by the developing egg-cell. In the case of *Dioon*, however, Chamberlain demonstrates a distinct communication between the jacket-cells and the egg in the form of cytoplasmic haustoria.

While the conclusions reached by these later writers are much more rational than any offered by previous investigators, their explanations do not seem entirely applicable to the conditions found in the Cupressineae. As I have stated above, in this latter group the wall separating the egg from the jacket-cells shows very little thickening, and I was unable to detect any cytoplasmic communication between them. I should also like to point out that in all of the Cupressineae, on account of the grouping of the archegonia in a single complex, many of the egg-cells do not come in contact with the jacket-cells at all. As clearly shown in Figs. 16, 17, 18, and 25 the egg-cells that are centrally situated are surrounded completely by neighbouring egg-cells. The cytoplasm of these centrally situated egg-cells showed very little difference in the character and quantity of food

granules from that of the egg-cells in contact with the nourishing jacket-cells. It therefore seems rational to assume that the manner of transference of food substances from egg-cell to egg-cell is the same as that from jacket-cell to egg-cell. In the thin walls separating the egg-cells from each other I was unable to detect any evidence of pits or perforations, and I therefore believe that all food substances carried into the egg are translocated in soluble form. In this connexion it may be worth while calling attention to the fact that a large vacuole, situated just below the egg-nucleus, is a constant character of the archegonium of all of the Cupressineae which I have examined (see Figs. 16, 20, and 25). It seems not improbable that the osmotic activities which these vacuoles must necessarily set up have much to do with the absorption of food substances in solution from the surrounding endosperm, and also from egg-cell to egg-cell. The sum total of the osmotic activities set up by twenty or more large, closely situated vacuoles must be considerable. In the Cupressineae, however, the archegonia and the manner of their grouping are highly specialized, and it is therefore not surprising to find the method of their nutrition somewhat different from such primitive types as the Cycads, *Ginkgo*, or the Abietineae.

Fertilization.

In the Podocarpeae (Coker, '02), Taxeae (Jäger, '99; Belajeff, '93; Robertson, '04; Lawson, '07), and Abietineae (Blackman, '98; Coulter and Chamberlain, '01; Ferguson, '01) the archegonia are few in number, and are not grouped closely together, but each is separated from its neighbour by prothallial tissue, and each possesses a small independent archegonial chamber leading to the neck. When the pollen-tube reaches one of these archegonial chambers its entire contents are discharged into the archegonium, and it is thus possible for only one archegonium to be fertilized by one pollen-tube—or in other words, only one of the two male gametes can be functional. The effect of this arrangement of the archegonia finds an expression in the form of the male gametes. For instance, in *Podocarpus* (Coker, '02) these structures are represented by a large male cell and a dwarfed male nucleus which does not function. In *Taxus* (Jäger, '99), and also in *Torreya taxifolia* (Coulter and Land, '05), there are two male cells organized, but one of them is much smaller than the other, and only the latter is functional. In *Cephalotaxus* (Lawson, '07) the male gametes are reduced to nuclei, both of which enter the archegonium, but only one unites with the egg-nucleus. In *Picea* and *Abies* (Miyake, '03), and also in *Pinus* (Ferguson, '01), the male cells are likewise represented only by nuclei, but in these forms the sperm nuclei are of unequal size, and it is only the larger one which is functional.

When we come to the Cupressineae, however, we find that the archegonia are grouped together in a single complex, with their necks exposed

at the base of a large common archegonial chamber. It is into this chamber that the contents of the various pollen-tubes are discharged. The archegonia being quite numerous, conditions are such that both male gametes from each tube may be functional. The effect of this arrangement likewise finds an expression in the form of the male gametes; for in all of the Cupressineae which have been investigated, namely, *Taxodium*, *Cryptomeria*, *Thuja*, *Libocedrus*, *Chamaecyparis*, *Cupressus*, and *Juniperus*, these structures take the form of two large male cells of equal size. This more primitive condition of the male cells is, however, not confined to this group, for we know that the male cells in *Sequoia* are very similar to those of the Cupressineae, but in this genus also the position of the archegonia in relation to the pollen-tubes is such as to permit of both male gametes functioning.

By the time the male cells and other structures of the tip of the pollen-tube have been discharged into the archegonial chamber, the neck-cells seem to disintegrate, leaving a free passage for the sperm-cells to enter the archegonia This was observed in both *Libocedrus* and *Thuja orientalis*. Pl. XXV, Fig. 17 is a section through the archegonial chamber at this time. One male cell may be seen about to enter an archegonium. The actual entrance of the male cell into the egg was not observed in *Libocedrus*, but in *Thuja* and *Chamaecyparis* a complete series of fertilization stages was obtained. In both of these forms but a single male cell was observed to enter the egg with an occasional stalk- or tube-nucleus. In no preparation was I able to find more than one male nucleus in the egg, as sometimes seems to be the case in *Taxodium* (Coker, '04). As many archegonia may thus be fertilized as there are male cells organized in the various pollen-tubes. Upon entering the archegonium the nucleus of the male cell escapes from its surrounding cytoplasm and advances immediately towards the egg-nucleus. The male cytoplasm advances in the same direction, but more slowly, as indicated in Pl. XXVI, Figs. 26 and 27. The egg-cytoplasm between the advancing male nucleus and the neck did not show that vacuole-like disturbance which is so characteristic of this stage in certain of the Abietineae and in *Cephalotaxus*, and this fact supports the statement made above that the tip of the pollen-tube does not enter the egg. As shown in Figs. 26, 27, and 28, the male nucleus presses closely into the membrane of the female, and the former, at this time, is less than half the size of the latter. There is not only a difference in the size of the sex nuclei, but the chromatic contents of the male are very unlike those of the female. The male chromatin is in the form of very small granules of uniform size, apparently supported on linin, forming a network of granular threads, as shown in Fig. 28. With the female nucleus, however, it was quite impossible to distinguish the true chromatin from the nucleoli and other irregularly shaped bodies which stained like chromatin and seemed to be closely associated with the latter. The two nuclei now enlarge considerably—the male more than the female—

and, as indicated in Fig. 29, their chromatic contents come to resemble each other more closely as to their structure. As shown in this figure, the membrane between the nuclei persists for some considerable time. During the process of fusion the granular, starch-containing cytoplasm from the male cell completely envelopes both nuclei. In the great difference in size of the sex nuclei at the time when they first come together, *Chamaecyparis*, *Thuja* (Land, '02), *Cupressus*, *Taxodium* (Coker, '04), and *Cryptomeria* (Lawson, '04) present conditions similar to those found in the Abietineae (Blackman, '98; Woycicki, '99; Coulter and Chamberlain, '01; Ferguson, '01; Miyake, '03; Murrill, '00), and in the Taxeae (Robertson, '04; Coulter and Land, '05; Lawson, '07). These conditions differ, however, from those found in *Sequoia* (Lawson, '04), for here the male nucleus is quite large at the time of its entrance into the egg.

In none of my preparations was I fortunate enough to find stages showing the formation of the first cleavage spindle, and I am therefore not in a position to give an account of the behaviour of the male and female chromosomes, or of the stage at which these elements lose their identity. From an examination of the early stages of the embryo in *Thuja*, *Libocedrus*, *Chamaecyparis*, and *Cupressus*, I can say without much doubt that this spindle is organized in the middle region of the archegonium in just about the same place where the fusion of sex nuclei took place. In this I agree with Land's ('02) observations on *Thuja occidentalis*, but cannot confirm Coker's ('04) account of *Taxodium*, where the fusing nuclei are reported as sinking to the base of the archegonium before the first division occurs.

The Embryo.

In *Libocedrus*, *Cupressus*, *Chamaecyparis*, and *Thuja* the first division of the fusion nucleus takes place near the centre of the archegonium. The two free nuclei resulting from this division come to lie very close together, and are enveloped in a granular, deeply staining sheath which seems to consist mainly of starch. In this condition the first two nuclei of the pro-embryo move towards the base of the archegonium, as indicated in Fig. 30. It is very probable that some of this starch comes from the male cytoplasm, but that all of it comes from this source seems quite as improbable, for by the time the free nuclei have reached the base of the archegonium the amount of starch surrounding the pro-embryo has increased enormously. Fig. 32 shows the pro-embryo of *Libocedrus* with the two daughter-nuclei of the first division, each enveloped in a sheath of starch. Fig. 31 shows a little older stage of the pro-embryo of *Thuja*, where the starch surrounding the two nuclei has increased to such an extent that it seems to completely fill the basal region of the archegonium in which the embryo is about to be developed. When the conditions shown in Fig. 31 has been reached, the first two nuclei of the embryo prepare for mitosis, and as shown in Fig. 33

they divide simultaneously. It will also be seen from this figure that the two spindles of this division lie one behind the other, with their long axes parallel with the long axis of the archegonium. The four daughter-nuclei resulting from this division are thus arranged in a single row, and not in the tetrad fashion as seems to be the case at this stage in *Taxodium* (Coker, '03). Land ('02) also figures a somewhat different position of the spindles of the second division in *Thuja occidentalis*. As only two or three preparations showed this stage of the embryo, I cannot say how constant or variable the position of the first four nuclei may be, but judging from the stage just before the third division, and also from a study of these stages in *Cryptomeria*, I am inclined to believe that there may be a considerable variation in the position of the free nuclei up to the time they arrange themselves in tiers and definite walls are formed between them.

Some time before and during the second division the archegonium becomes differentiated into two distinct regions, and this seems to be brought about by the sinking of all the starch, 'proteid-vacuoles,' and other food substances into the basal region of the fertilized egg in very much the same manner as that which I have reported for *Cephalotaxus* (Lawson, '07). It is only this nutritive or basal region of the archegonium which now concerns the development of the embryo, and this is indicated in Figs. 31, 33, 34, and 35.

No sooner are the first four nuclei organized than they prepare for the third mitosis. This latter division is also simultaneous, and it results in the formation of eight free nuclei in the pro-embryo. From Land's ('02) account of the embryo of *Thuja*; Coker's ('03) account of *Taxodium*, and my own observations on *Cryptomeria*, *Thuja*, *Libocedrus*, and *Cupressus*, it seems that the organization of eight free nuclei in the pro-embryo, before the formation of cell-walls, is a constant feature of the Cupressineae.

Fig. 34 shows a stage just previous to the formation of the first cell-walls of the embryo. These walls are obviously formed in the same manner as they are in *Cryptomeria* (Lawson, '04). The spindles of the first two divisions disappear entirely after the organization of the daughter-nuclei, but the continuous fibrils of the spindles of the third division persist until membranes are formed from them midway between the nuclei. The cells of the pro-embryo, as a result of these divisions, are arranged in three distinct tiers—or rather two tiers of cells and one tier of free nuclei, for the upper tier or rosette are not surrounded by walls. The middle tier of cells very soon begin to elongate, and are thus easily identified as suspensors. The end tier of cells or embryo cells proper show very little enlargement, and no more merismatic activity whatever until they have been carried for a considerable distance into the endosperm tissue by the enormous elongation of the suspensors. In this regard *Libocedrus* and other Cupressineae are very unlike that of *Cephalotaxus*, for in this latter genus the embryo cells

become very numerous before the suspensors elongate (Lawson, '07). In this latter case, however, the work of the suspensors in carrying the embryo into the endosperm is supplemented by a penetrating cap formed from the tip cells of the embryo.

The further development of the embryo in *Libocedrus* agrees in all essential details with that which I have described for *Cryptomeria* (Lawson, '04), and closely resembles the account given by Land ('02) in the case of *Thuja*. Fig. 36 shows an embryo of *Libocedrus* with the middle tier of suspensor cells considerably elongated, and a group of embryo cells proper at the tip. The nuclei of the rosette may also be seen in the cytoplasm of the archegonium above. Fig. 37 represents a still later stage in the development of the suspensors.

Summary and Conclusion.

From investigations on the pollen of *Taxodium*, *Cryptomeria*, *Thuja*, *Libocedrus*, *Chamaecyparis*, *Cupressus*, and *Juniperus*, it seems that the microspores of the Cupressineae are characterized by the absence of vestigial prothallial cells or of nuclei representing such cells.

The mature microspore consists of two cells, containing respectively the generative and tube-nuclei.

With the exception of *Cupressus* and *Juniperus*, the first division of the microspore, which gives rise to the generative and tube-nuclei, takes place before pollination.

By the time the pollen-tube has penetrated the nucellar tissue the generative nucleus enlarges and divides. As a result of this division the body-cell and stalk-nucleus are organized.

The growth of the pollen-tube is almost in a straight line towards the archegonial chamber, and as it advances the body-cell and stalk- and tube-nuclei are always found at the tip.

When the archegonial chamber has been reached the body-cell divides, giving rise to two large male cells of equal size.

The case reported by Juel for *Cupressus Goweniana*, where the body-cell gives rise to a cell complex, is regarded as an abnormality.

The number of megaspore mother-cells formed may vary from one to three, each of which divides twice. Only one of the resulting megaspores germinates.

The presence of a tapetum surrounding the megaspore seems to be characteristic of the group, but it varies considerably as to the extent of its development in the different genera studied.

The functioning megaspore first enlarges, and this is followed by rapid free nuclear division. A number of vacuoles now make their appearance, but as the spore enlarges these flow together, forming a very large central

vacuole which forces the cytoplasm and numerous free nuclei to the periphery. The parietal layer of cytoplasm consists of a mere film in which the free nuclei are distributed at intervals.

Free nuclear division now proceeds, and the amount of cytoplasm also increases. It is believed that the osmotic activities set up by the central vacuole have much to do with the absorptions of food materials from the surrounding nucellar tissue.

As the amount of cytoplasm increases, free nuclear division ceases, and walls are formed between the nuclei. The primary prothallial cells thus formed are open and exposed to the sap of the vacuole on the inner side. The primary cells now elongate rapidly, and by their inward growth the space occupied by the central vacuole is eventually closed. During their growth numerous cross-walls are formed in the primary cells, but those on the inner side always remain open until the vacuole vanishes.

A poorly developed megaspore membrane is characteristic of all the Cupressineae examined.

The archegonia vary in number from six or eight to twenty-four, and are always grouped closely together in a single complex. There is a common large archegonial chamber, at the base of which the necks of the archegonia lie closely clustered together. A single layer of jacket-cells surrounds the archegonial complex.

In none of the Cupressineae is there a ventral canal-*cell* formed. This structure is represented only by a nucleus.

The contents of the various pollen-tubes become discharged into the archegonial chamber, and as the archegonia are numerous it is thus possible for both male cells from each pollen-tube to function. One pollen-tube may fertilize two separate archegonia.

As a rule one male cell enters the egg, and its nucleus slips from its cytoplasm and unites with the egg-nucleus. When the sex nuclei first come in contact with each other the female is three or four times the size of the male.

The first spindle of the sporophyte is organized near the middle of the archegonium, in the same place where the fusion of the sex nuclei occurred. After the first division the two free nuclei of the pro-embryo become enveloped in a sheath of starch, and move towards the base of the archegonium. In this position they divide, and this is immediately followed by a third division resulting in the formation of eight free nuclei before any cell-walls are formed.

The cells of the pro-embryo become arranged in three tiers—the upper forming the rosette, the middle tier which develops into the suspensors, and the group of cells at the tip which form the embryo proper.

Considering the state of development of all the various vestigial structures associated with the gametophytes, and comparing them with

other Conifers, the Cupressineae cannot be regarded as a very primitive group. They certainly do not present as many primitive characters as the Abietineae, but on the other hand they are more primitive than *Cephalotaxus*.

Literature Cited.

Arnoldi, W. ('00): Beiträge zur Morphologie der Gymnospermen: III. Embryogenie von *Cephalotoxus Fortunei*. Flora, lxxxvii, p. 46, 1900.

——— ('00): Beiträge zur Morphologie einiger Gymnospermen: I. Die Entwicklung des Endosperms bei *Sequoia sempervirens*. Bull. Soc. Imp. Nat. Moscou, 1900.

——— ('00): Beiträge zur Morphologie und Entwicklungsgeschichte einiger Gymnospermen: II. Ueber die Corpuscula und Pollenschläuche bei *Sequoia sempervirens*. Bull. Soc. Imp. Nat. Moscou, 1900.

——— ('01): Beiträge zur Morphologie einiger Gymnospermen: V. Weitere Untersuchungen über die Embryologie in der Familie der Sequoiaceen. Bull. Soc. Imp. Nat. Moscou, 1901.

Belajeff, W. ('93): Zur Lehre von dem Pollenschlauche der Gymnospermen. Ber. der Deutsch. Bot. Gesell., ii, p. 196.

Blackman, V. H. ('98): On the Cytological Features of Fertilization and Related Phenomena in *Pinus silvestris*, L. Phil. Trans. Roy. Soc., cxc, p. 395.

Chamberlain, C. J. ('06): The Ovule and Female Gametophyte of *Dioon*. Bot. Gaz., xlii, p. 321, 1906.

Coker, W. C. ('02): Notes on the Gametophytes and Embryo of *Podocarpus*. Bot. Gaz., xxxiii, p. 89.

——— ('03): The Gametophytes and Embryo of *Taxodium*. Bot. Gaz., xxxvi, pp. 1–27 and 114–40.

——— ('04): On the Spores of Certain Coniferae. Bot. Gaz., xxxviii, p. 206, 1904.

Coulter, J. M., and Chamberlain, J. C. ('01): Morphology of Spermatophytes, Part I. New York, 1901.

Coulter, J. M., and Land, W. J. G. ('05): Gametophytes and Embryo of *Torreya taxifolia*. Bot. Gaz., xxxix, p. 161, 1905.

Dixon, H. N. ('94): Fertilization in *Pinus silvestris*. Ann. Bot., viii, p. 21.

Ferguson, M. C. ('01): The Development of the Pollen-tube and the Division of the Generative Nucleus in certain species of *Pinus*. Ann. Bot., xv, p. 193.

——— ('01): The Development of the Egg and Fertilization in *Pinus Strobus*. Ann. Bot., xv, p. 435.

Goroschankin, J. ('88): Zur Kenntniss der Corpuscula bei den Gymnospermen. Bot. Zeit., xli, p. 825.

Hirase, S. ('95): Études sur la fécondation et l'embryogénie du *Ginkgo biloba*. Jour. Coll. Sci. Imp. Univ. Tokyo, viii, p. 307.

Ikeno, S. ('96): Das Spermatozoid von *Cycas revoluta*. Bot. Mag. Tokyo, x, p. 367.

Jäger, L. ('99): Beiträge zur Kenntniss der Endospermbildung und zur Embryologie von *Taxus baccata*. Flora, lxxxvi, p. 241.

Jeffrey, E. C. ('03 and '05): Comp. Anatomy and Phylogeny of the Coniferales. Part I, *Sequoia*; Part II, Abietineae. Mem. Boston Soc. Nat. Hist., vols. 5 and 6.

Juel, H. O. ('00): Beiträge zur Kenntniss der Tetradentheilung. Jahrb. f. wiss. Bot., xxxv, p. 626, 1900.

——— ('04): Ueber den Pollenschlauch von *Cupressus*. Flora, xciii, p. 56, 1904.

Land, W. J. G. ('02): A Morphological Study of *Thuja*. Bot. Gaz., xxxvi, p. 249.

Lawson, A. A. ('04): The Gametophytes, Archegonia, Fertilization, and Embryo of *Sequoia sempervirens*. Ann. Bot., xviii, no. lxix, p. 1, 1904.

LAWSON, A. A. ('04): The Gametophytes, Fertilization, and Embryo of *Cryptomeria japonica*. Ann. Bot., xviii, no. lxxi, 1904.
——— ('07): The Gametophytes, Fertilization, and Embryo of *Cephalotaxus drupacea*. Ann. Bot., xxi, no. lxxxi, 1907.
MIYAKE, K. ('03): On the Development of the Sexual Organs and Fertilization of *Picea excelsa*. Ann. Bot., xvii, p. 66.
——— ('03): Contribution to the Fertilization and Embryogeny of *Abies balsama*. Beihefte zum Bot. Cent., xiv, p. 134, 1903.
MURRILL, W. A. ('00): The Development of the Archegonium and Fertilization in the Hemlock Spruce (*Tsuga canadensis*). Ann. Bot., xiv, p. 583.
ROBERTSON, A. ('04): Spore formation in *Torreya Californica*. New Phyt., iii, p. 133, 1904.
——— ('04): Studies in the Morphology of *Torreya Californica* New Phyt., iii, p. 205, 1904.
SHAW, W. R. ('96): Contribution to the Life-history of *Sequoia sempervirens*. Bot. Gaz., xxi, p. 332.
SOKOLOWA, MLLE C. ('90): Naissance de l'endosperme dans le sac embryonnaire de quelques Gymnospermes. Bull. Soc. Imp. Nat. Moscou, 1890 (1891), p. 446.
STRASBURGER, E. ('72): Die Coniferen und Gnetaceen, 1872.
——— ('79): Die Angiospermen und die Gymnospermen, 1879.
——— ('84): Neue Untersuchungen über die Befruchtungsvorgänge bei den Phanerogamen als Grundlage für eine Theorie der Zeugung. Jena, 1884.
——— ('92): Ueber das Verhalten des Pollens und die Befruchtungsvorgänge bei den Gymnospermen. Hist. Beit., iv, p. 1, 1892.
STOPES, M. C., and FUJII, K. ('06): The Nutritive Relations of the surrounding Tissues to the Archegonium in Gymnosperms. Beihefte zum Bot. Cent., Bd. xx, Heft 1, 1906.
THOMSON, R. B. ('05): The Megaspore-membrane of the Gymnosperms. Univ. Toronto Studies, Biol. Series, iv, 1905.
WEBBER, H. J. ('97): Peculiar Structure in the Pollen-tube of *Zamia*. Bot. Gaz., xxiii, p. 453.
——— ('97): The Development of the Antherozoids of *Zamia*. Bot. Gaz., xxiv, p. 16.
——— ('97): Notes on the Fecundation of *Zamia* and the Pollen-tube Apparatus of *Ginkgo*. Bot. Gaz., xxiv, p. 225.
——— ('01): Spermatogenesis and Fecundation of *Zamia*. U.S. Dept. Agri. Bur. of Plant Industry. Bull., ii, 1901.
WOYCICKI, Z. ('99): Fertilization in the Coniferae. Warsaw, 1898. Reviewed in Journ. Roy. Micro. Soc., p. 482, 1900.

EXPLANATION OF FIGURES IN PLATES XXIV–XXVI.

Illustrating Dr. Lawson's Paper on the Cupressineae.

All figures were drawn with the aid of the camera lucida. The following oculars and objectives were used:—

Figs. 1, 2, 8, Zeiss. oc. 6, obj. 16 mm.
Figs. 3, 33, 35, Zeiss. oc. 2, obj. 3 mm.
Figs. 4, 5, 6, 7, 28, Zeiss. oc. 1, obj. $\frac{1}{12}$ oil imm.
Figs. 9, 10, 11, 12, 29, Zeiss. oc. 2, obj. $\frac{1}{12}$ oil imm.
Figs. 13, 15, Zeiss. oc. 4, obj. 16 mm.
Figs. 14, 21, 22, 23, 24, Zeiss. oc. 4, obj. $\frac{1}{12}$ oil imm.
Fig. 27, Zeiss. oc. 3, obj. $\frac{1}{8}$ oil imm.
Figs. 33, 35, Zeiss. oc. 2, obj. $\frac{1}{8}$ oil imm.
Figs. 16, 17, 18, 19, 20, 25, 26, 30, 31, 33, 34, Zeiss. oc. 6, obj. $\frac{1}{8}$ oil imm.

Fig. 1. A longitudinal section through the upper part of the nucellus showing a young pollen-tube. *Thuja orientalis*. May 12.

Fig. 2. The same at a later stage showing at least four pollen-tubes. May 22.

Fig. 3. A longitudinal section through the end of a pollen-tube showing the body-cell and tube-nucleus. *Thuja orientalis.* May 27.

Fig. 4. The contents of the tip of an older pollen-tube showing the body-cell and stalk- and tube-nuclei. *Thuja orientalis.* May 22.

Fig. 5. A section of one of the male cells just before fertilization. May 27.

Fig. 6. A section of the body-cell some time before the final division which gives rise to the two male cells. *Libocedrus decurrens.* May 31.

Fig. 7. Two male cells some time before fertilization. *Libocedrus decurrens.* June 1.

Fig. 8. A longitudinal section of a young megasporangium showing two megaspore mother-cells. *Libocedrus decurrens.* March 22.

Fig. 9. A section of two megaspore mother-cells more highly magnified. *Libocedrus decurrens.* March 23.

Fig. 10. A section of four megaspores just after the first division of the mother-cells. *Libocedrus decurrens.* March 23.

Fig. 11. A section showing six of the eight megaspores after the second division. *Libocedrus decurrens.* March 23.

Fig. 12. A section of a megaspore some time after germination showing numerous free nuclei in process of division. *Libocedrus decurrens.* April 13.

Fig. 13. A section through the lower half of a young female prothallium showing the large central vacuole and parietal layer of cytoplasm in which numerous free nuclei lie embedded. *Libocedrus decurrens.* May 8.

Fig. 14. A section of the parietal layer of cytoplasm more highly magnified and taken from the stage shown in Fig. 13. *Libocedrus decurrens.* May 8.

Fig. 15. A longitudinal section of the lower half of a young female prothallium showing the inward growth of the primary prothallial cells and the consequent diminution in the size of the central vacuole. The nuclei are much more numerous at the periphery of the cells exposed to the fluid of the vacuole. *Thuja orientalis.* May 11.

Fig. 16. A longitudinal section through an archegonial complex showing the single layer of jacket-cells surrounding the whole group. *Thuja orientalis.* May 30.

Fig. 17. A longitudinal section of an archegonial complex showing a portion of the archegonial chamber above, into which the contents of the pollen-tubes have been discharged. *Thuja orientalis.* May 30.

Fig. 18. A cross-section of an archegonial complex showing the common jacket surrounding twenty-three archegonia. *Thuja orientalis.* May 22.

Fig. 19. A longitudinal section of an archegonium showing the central nucleus undergoing division. *Thuja orientalis.* May 30.

Fig. 20. A longitudinal section of an archegonium showing the position of the ventral canal-nucleus and the egg-nucleus. *Thuja orientalis.* May 30.

Fig. 21. A more highly magnified section of the spindle which gives rise to the egg-nucleus and ventral canal-nucleus. *Thuja orientalis.* May 30.

Fig. 22. A section of the spindle which gives rise to the ventral canal-nucleus and egg-nucleus. *Libocedrus decurrens.* June 1.

Fig. 23. A more highly magnified section showing the ventral canal-nucleus and egg-nucleus just after the division of the central cell. *Thuja orientalis.* May 30.

Fig. 24. A later stage of the same showing the absence of any trace of a membrane between the two nuclei. May 30.

Fig. 25. A longitudinal section through an archegonial complex just before fertilization. *Libocedrus decurrens.* June 6.

Fig. 26. An archegonium showing the fusion of the male and female nuclei. *Thuja orientalis.* May 30.

Fig. 27. A more highly magnified view of a similar stage showing the presence of the male cytoplasm above the fusing nuclei, and also the presence of numerous so-called 'proteid-vacuoles.' May 30.

Fig. 28. A section of the male and female nuclei in process of fusion showing their relative size and the difference in the structure of their chromatic contents *Thuja orientalis.* May 30.

Fig. 29. A later stage of the same showing the increase in size of the male nucleus and the similarity in the structure of the chromatic contents of both nuclei. May 30.

Fig. 30. A longitudinal section of an archegonial complex showing three pro-embryos with a zone of starch surrounding each. *Libocedrus decurrens.* June 1.

Fig. 31. A pro-embryo showing the accumulation of starch and other granular substances in the basal region of the archegonium. *Thuja orientalis.* May 30.

Fig. 32. A pro-embryo after the first division showing the two nuclei surrounded by a dense zone of starch. *Libocedrus decurrens.* June 1.

Fig. 33. A later stage of the pro-embryo with the two free nuclei undergoing division. *Libocedrus decurrens.* June 1.

Fig. 34. A later stage of the pro-embryo with eight free nuclei. *Thuja orientalis.* May 30.

Fig. 35. A pro-embryo of eight free nuclei with no cell-walls separating them. *Libocedrus decurrens.* June 1.

Fig. 36. An embryo showing the elongation of the middle tier of cells to form the suspensors. *Libocedrus decurrens.* June 1.

Fig. 37. A later stage of the same showing the suspensors more fully developed. *Libocedrus decurrens.* June 2.

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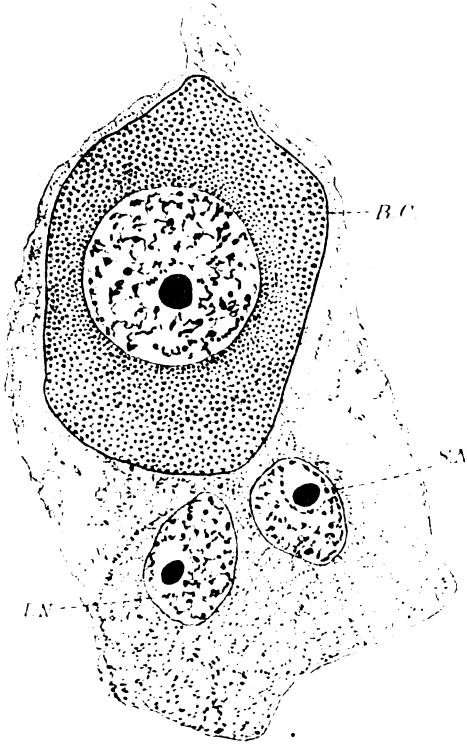

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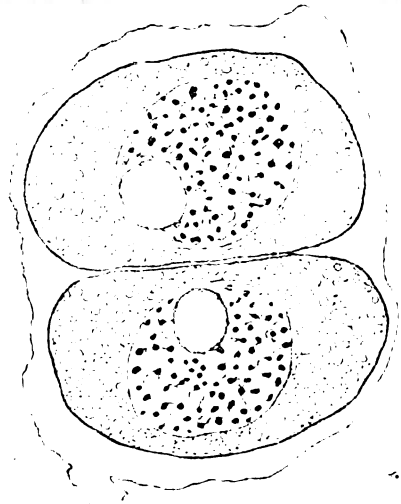

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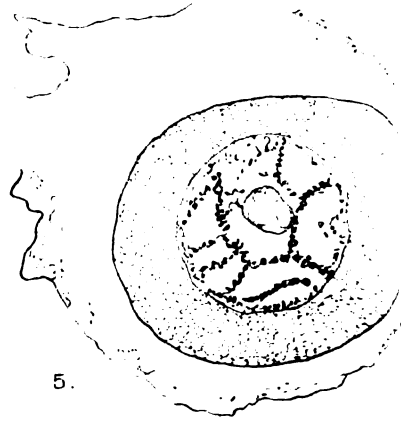

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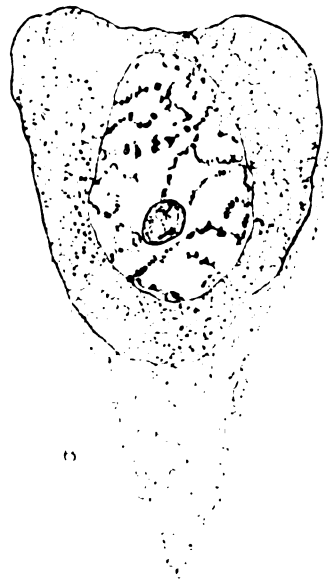

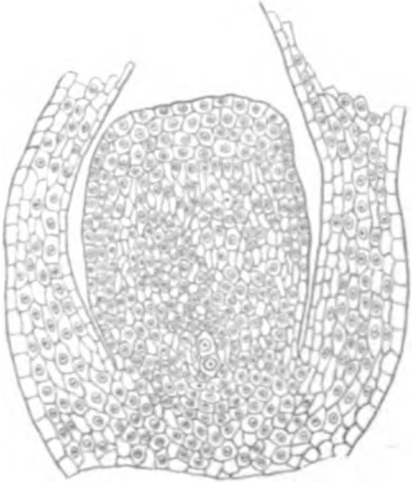

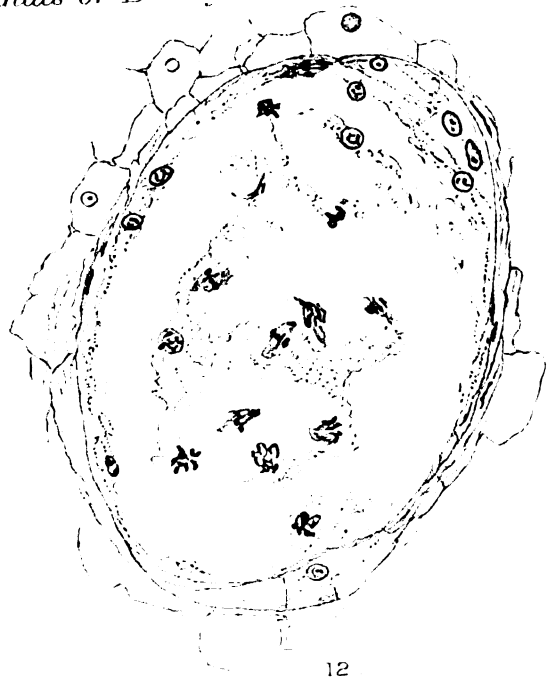

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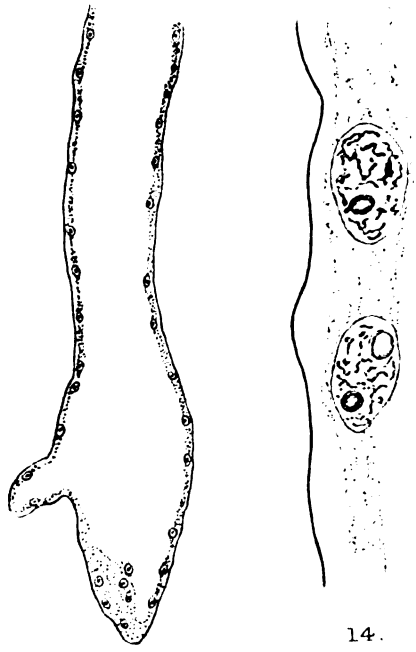

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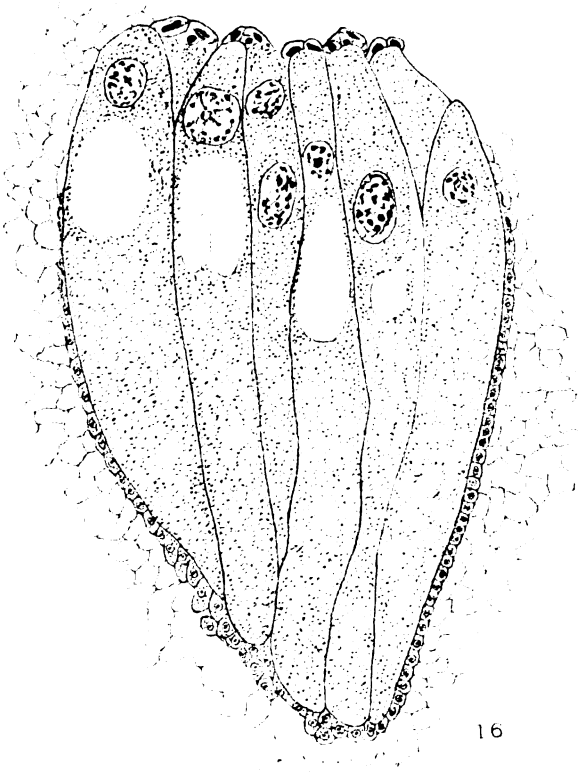

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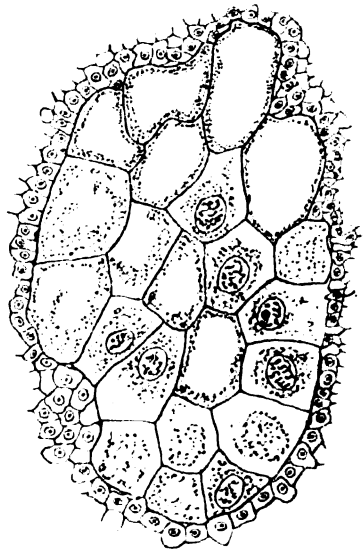

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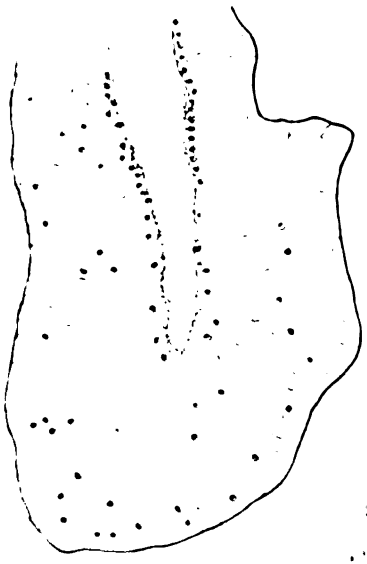

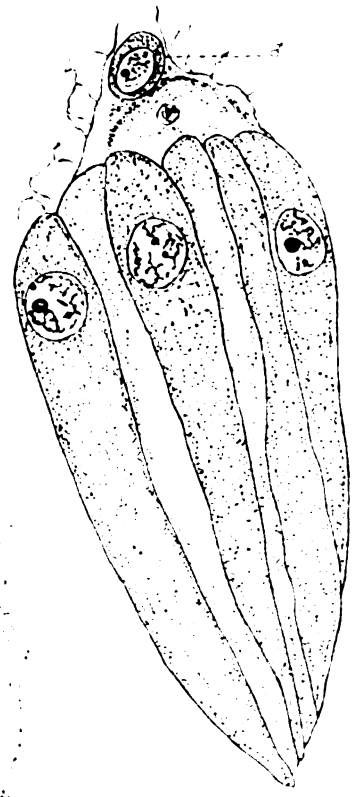

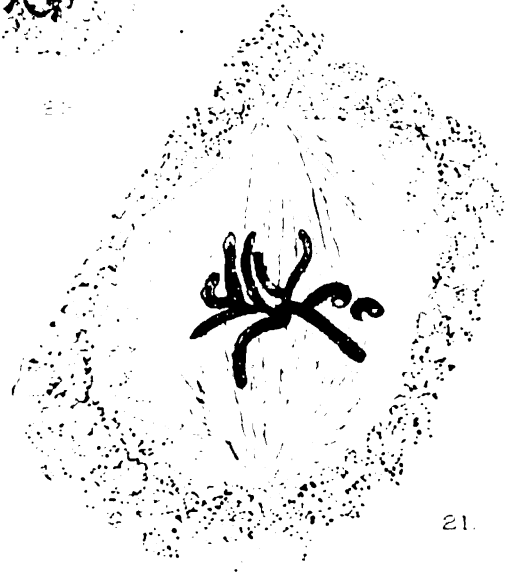

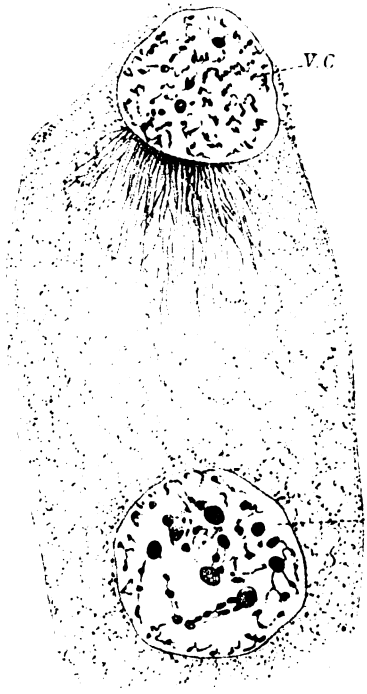

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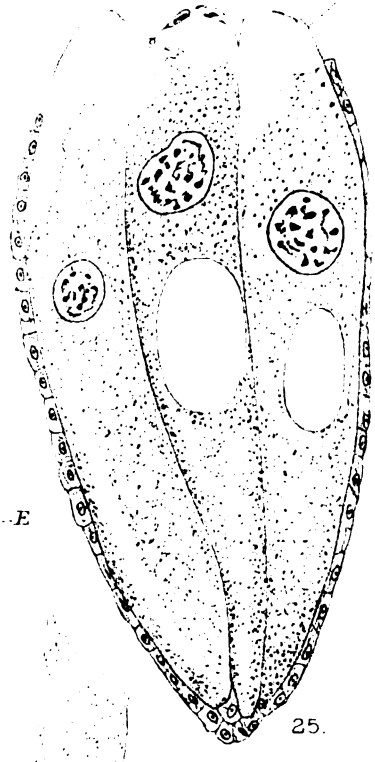

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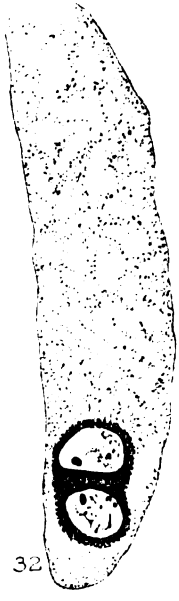

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NOTES.

NOTE ON THE PALAEOZOIC SEEDS, TRIGONOCARPUS AND POLYLOPHOSPERMUM.—In their detailed and very interesting paper on the palaeozoic seed-genus *Trigonocarpus*[1], Dr. Scott and Mr. Maslen include a description of a new species with which they have done me the honour to associate my name. The chief interest attaching to the still imperfectly known *T. Oliveri* depends on the resemblance which it presents to the *Polylophospermum stephanense* of Brongniart.

The particular feature which the authors find these two seeds to possess in common is an inverted chalazal cup or circular ridge at the base, enclosing the stalk by which the seed must have been attached to the structure which bore it.

My excuse for writing the present note, before the authors have completed their labours, is to mention a character, hitherto undescribed, in which *Polylophospermum* approaches the type-species *Trigonocarpus Parkinsoni* (*Trigonocarpon olivaeforme*), a character that may prove of some taxonomic importance when the relations of these seeds are considered.

Through the courtesy of Professor E. Bureau of the Muséum d'Histoire Naturelle it was possible, during the course of a visit to Paris in April, 1905, to consult the fossil slides in the collection of the late Monsieur Renault.

Among these were several sections of *Polylophospermum*, which contribute one or two points hitherto unrecorded. Transverse sections across the body of the seed show the hexagonal outline, familiarized by Brongniart's original sketch[2], with six principal acute ridges along the salient angles of the sclerotesta, and a like number of lower and less acute secondary ribs on the flat, prismatic faces. In several of these specimens considerable traces of sarcotesta may be recognized, extending both between the ribs and also beyond them; whilst, embedded in the tissue of the sarcotesta, immediately outside the points of the secondary ribs, little vascular bundles are found to occur. In some cases these bundles are distorted and flattened in the tangential plane, but their presence in the position described was confirmed in a sufficient number of cases to remove all reasonable doubt as to the accuracy of the observation. On the other hand, no bundles were detected in the sarcotesta in the neighbourhood of the principal ridges.

These facts are epitomized in Fig. 1, A, which is based primarily on the preparation numbered 9306[3]. Another preparation from the same series contained a longitudinal section of the chalazal end of a seed showing one of these vascular bundles running in the sarcotesta for a distance of about 5 mm. (Fig. 1, B, *v. b*).

From these observations it would appear that *Polylophospermum stephanense* possessed a sarcotestal vascular system consisting of bundles running in the same radii as the secondary ridges; on the other hand, the principal ridges seem to have been unaccompanied by bundles.

[1] Ann. of Bot., vol. xxi, p. 89.

[2] A. Brongniart, Rech. sur les graines foss. silicifiées, Pl. C, Fig. 7.

[3] Other preparations illustrating the point are 9315, 9317.

Furthermore, each principal ridge is characterized by a longitudinal crack in its plane of symmetry, proceeding from the furrow on the interior surface of the sclerotesta.

Hence a close general agreement obtains between the two seeds (*Trigonocarpus Parkinsoni* and *Polylophospermum stephanense*)—(1) in the differentiation of the ridges; (2) in the presence of a sarcotestal vascular system; (3) in the correlation with the principal and secondary ridges of radial cracks and vascular bundles, respectively.

Under these circumstances it is of interest that a seed should have come to light (*T. Oliveri*), showing transitional characters between the two genera.

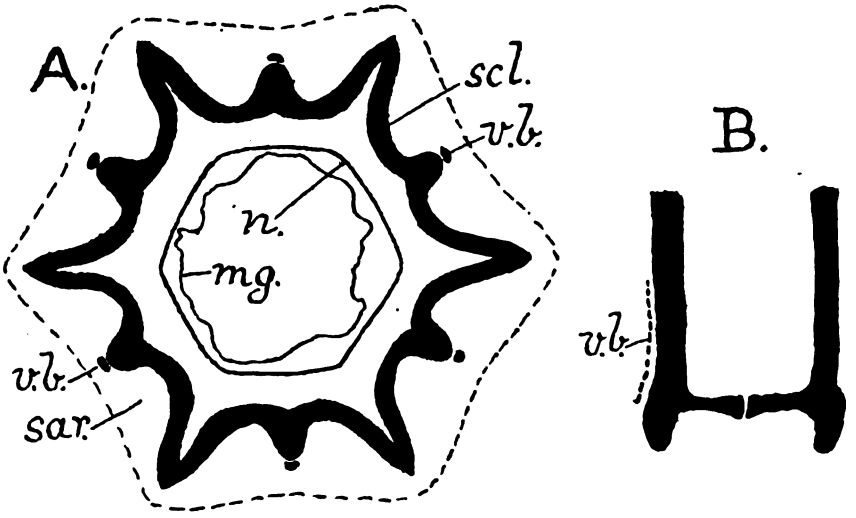

Fig. 1. *Polylophospermum stephanense.*

A, transverse section of seed showing the position of the vascular bundles of the sarcotesta and their relation to the ridges. *n.* nucellus; *mg.* megaspore membrane; *sar.* sarcotesta; *scl.* sclerotesta; *v.b.* vascular bundles. × 8.

B, longitudinal section of the chalazal end of a seed. *v.b.* vascular bundle. × 4.

Before closing this note there is one point I would raise before it is too late. In Pt. I of their paper[1] the authors show a tendency to adopt the term *Stephanospermeae* as the group-name for the seeds showing a general agreement in structure with *Trigonocarpus*. My plea is that *Stephanospermeae* be abandoned in favour of the much more appropriate *Trigonocarpeae*. The only justification for the term *Stephanospermeae* is that at the time it was proposed it embodied the name of the seed the anatomy of which had been investigated in rather more detail, perhaps, than was the case with any other member of the group. To-day that qualification is no longer valid. Moreover, *Trigonocarpus* is the Coal-measure seed *par excellence*, and has been the subject of numerous memoirs and references scattered over the palaeo-botanical literature of nearly a century. It is widely distributed, and is known under both forms of preservation; whilst the casts and petrifactions have been correlated in detail. Hence on every ground *Trigonocarpus* has pre-eminent claims to give its name to the group.

F. W. OLIVER.

[1] Scott and Maslen, loc. cit.

THE SUPERNUMERARY POLLEN-GRAINS OF FUCHSIA.—In the great majority of Gymnosperms and Angiosperms four pollen-grains are produced from each pollen mother-cell.

Exceptions to this rule are, however, not wanting, and Coulter and Chamberlain [1] record (largely from Wille's observations) no less than twenty-three species in which a greater or less number of pollen-grains than four have been seen to arise from one mother-cell. To this list other names might be added, but I will only mention here that of *Aesculus Hippocastanum*, in which I have several times observed six microspores lying within one mother-cell wall. The manner in which this deviation from the normal number of microspores is produced has only been investigated in a very few instances. Apart from certain hybrids, such as *Syringa rothomagensis* (Juel [2]) and *Bryonia* (Tischler [3]), which form a rather special case, there are, so far as I am aware, only two forms in which the cytological processes underlying the production of an abnormal number of pollen-grains have been worked out. Thus in the Cyperaceae (*Heleocharis*, *Carex acuta*) the work of Elfving [4], Wille [5,6], Strasburger [7], and Juel [8] has shown that, whilst the mother-cell nucleus divides twice in the usual way, three of the nuclei thus formed degenerate, and only one microspore (surrounded by the thickened mother-cell wall) is finally developed from the mother-cell. Again, in *Hemerocallis fulva* Strasburger [9] and Juel [10] have shown that, during the anaphase of the first and second divisions of the mother-cell nucleus, the chromosomes are often irregularly distributed upon the spindle so that some of them, either singly or in groups, do not reach the poles, but become separated from their fellows, and each such group may give rise to a distinct nucleus. Each nucleus thus formed usually becomes the centre of a separate cell, so that a larger number of daughter-cells than four is frequently produced from a single mother-cell. Fullmer [11] (like Tangl [12] at an earlier date) is inclined to attribute at least some of the supernumerary microspores of *Hemerocallis* to the subsequent division of one or more of the tetrad nuclei, but Juel [13] entirely repudiates this suggestion. I have recently examined the pollen development of the ordinary greenhouse *Fuchsia* [14], and I can fully confirm previous writers with regard to the occurrence of supernumerary pollen-grains in this plant.

As long ago as 1850 Wimmel [15] called attention to the irregularities in the number and size of the pollen-grains produced from the mother-cell of *Fuchsia*, and in 1886

[1] Morphology of the Angiosperms, 1903, p. 125.
[2] Pringsheim's Jahrb. f. wiss. Bot., Bd. xxxv, 1900, p. 638.
[3] Berichte d. deutsch. Bot. Gesellsch., Bd. xxiv, 1906, p. 83.
[4] Jenaische Zeitschr. f. Naturwiss., Bd. xiii, 1879.
[5] Christiania Videnskabs-Selskabs Forhandlinger, 1882, No. 16.
[6] 'Ueber die Entwickelungsgeschichte der Pollenkörner der Angiospermen,' &c., 1886, Christiania, p. 43.
[7] 'Neue Untersuchungen über den Befruchtungsvorgang bei den Phanerogamen,' &c. Jena, 1884, p. 11.
[8] Juel, l. c., p. 649.
[9] Archiv. f. Mikrosk. Anat., Bd. xxi, 1882, p. 497.
[10] Pringsh. Jahrb. f. wiss. Bot, Bd. xxx, 1897, pp. 205-6.
[11] Bot. Gazette, vol. xxviii, 1899, p. 81.
[12] Denkschr. d. Kais. Akad. d. Wiss., Bd. xlv. Wien, 1882, p. 73.
[13] Pringsh. Jahrb. f. wiss. Bot., Bd. xxxv, 1900, p. 646.
[14] The pollen development of several different species of *Fuchsia* will be described in the full paper.
[15] Bot. Zeit., Bd. viii, 1850.

Wille [1] counted five, six, seven, and even fourteen microspores arising from a single mother-cell.

Moreover, Wille offers an explanation of the manner in which these additional pollen-grains are formed. Thus he writes, 'In dem Falle, wo bei *Fuchsia*, sp., 7 Zellen entstanden waren, konnte ich nicht darüber im Zweifel sein, dass dies daher kam, dass drei der Zellkerne der Tetrade noch sich einmal getheilt hatten, ehe die Cellulosequerwände ausgebildet waren, während der Vierte ungetheilt blieb.' Where five or six microspores were produced he believed the explanation to be similar. In the case of the higher numbers (14) he was unable to follow the cell-divisions, and is uncertain whether the additional pollen-grains are due to secondary divisions of one pollen mother-cell alone, or whether they are to be derived from two or more primitive mother-cells (Urmutterzellen) which have not become separated from one another in the usual manner.

I have frequently counted six, eight, and ten microspores lying within one mother-cell wall of this plant.

A study of the nuclear divisions of the mother-cell has shown that the high number of pollen-grains produced is due to the occurrence of irregularities in the distribution of the chromosomes during the anaphase of division, quite comparable with those described by Juel in *Hemerocallis fulva*, and no facts have been found to support Wille's explanation.

The prophase of the first division appears to take place in quite the normal manner, but during the anaphase it is seen that the numerous, small chromosomes frequently move very unevenly towards the spindle poles, and some, either singly or in groups, lag behind the rest and often become entirely cut off from the two main chromosome groups. Usually these separated chromosomes give rise to distinct nuclei, which vary in size according to the number of chromosomes they receive. A few cases were, however, observed in which the scattered arrangement of the chromosomes appears to have been such, that separate nuclear walls were not formed round each chromosome or group of chromosomes, but one lobed nuclear wall was produced which enclosed all the scattered chromosomes within its embrace [2]. It also occasionally happens that the separated chromosomes fail to become organized into distinct nuclei with nuclear walls, and in that case they appear to degenerate in the cytoplasm without taking any further share in pollen development. These cases are, however, infrequent, and in the majority of instances each detached chromosome or chromosome-group gives rise to a separate nucleus.

During the second meiotic division the small, as well as the large, nuclei undergo karyokinesis and produce a distinct spindle.

Often only two chromosomes can be seen to occupy the equator of a miniature spindle, and in some cases I believe only a single chromosome was present.

The second division of the mother-cell nucleus is much more regular than the first, and up to the present I have not found that any of the supernumerary nuclei originate at this stage. The nuclear divisions of the mother-cell which have been

[1] Ueber d. Entwickelungsgesch. d. Pollenkörner, &c., 1886, pp. 60–1.

[2] In some cases the lobed nuclei almost suggest the existence of amitotic divisions. After a careful comparison of all the preparations showing the phenomenon, I believe, however, that the interpretation given above is the correct one.

described above are followed by cell divisions. Thus the supernumerary pollen-grains are produced by the organization of distinct cells round all the nuclei, whether these have been formed in the regular course of mitosis, or by the isolation of irregularly distributed chromosomes.

I can find no evidence of the existence of secondary divisions of the cells such as Wille described, nor do my observations give any support to this author's suggestion that a fusion (or non-separation) of primitive mother-cells (Urmutterzellen) might occur in those cases in which the additional microspores were very numerous.

A definite relation appears to exist between the number of chromosomes entering into a nucleus, the size of the nucleus, and the size of the cell produced.

It may be further added that the small cells, with only a few chromosomes entering into the composition of their nuclei, develop pollen-walls, which differ neither in structure nor in chemical composition (viz. they give the same staining reactions) from those surrounding the pollen-grains which have received the normal number of chromosomes.

It will be seen that these facts have an interesting bearing upon the theory of the localization of specific characters in particular chromosomes, but the discussion of this matter must be left for my full paper upon the pollen development of *Fuchsia* and some other plants.

RUDOLF BEER.

CONTRIBUTIONS TO THE CYTOLOGY OF HUMARIA RUTILANS FR.

(Preliminary Note.)—*Humaria rutilans* is a small orange Discomycete occurring in abundance on sandy soil. It possesses exceptionally large nuclei, the nucleus of the uninucleate ascus measuring about $14\,\mu \times 9\,\mu$.

The ascocarp originates as a tangle of septate hyphae, each cell containing one or a few nuclei. Sexual organs are not differentiated. Very early a sheath of rather thick-walled cells can be distinguished, and within this ramify numerous hyphae, growing upwards till, while the ascocarp is still quite minute, paraphyses and subsequently asci appear. At and rather before this stage two sorts of hyphae can be distinguished in the hypothecium by the size of their nuclei, though they do not otherwise differ. The larger nuclei are about twice the size of the smaller, and are formed by the *fusion of these in pairs*. This would appear to constitute a process of reduced fertilization, or apogamy, quite comparable to that observed in the prothallus of *Nephrodium*, and representing a stage in the reduction of sexuality more advanced than that found in *Humaria granulata*, where an ascogonium is organized and the female nuclei fuse in pairs. The hyphae containing the larger, or fusion-nuclei, may thus be regarded as sporophytic, the others as gametophytic.

Asci arise from the sporophytic or ascogenous hyphae. The hypha, on reaching the subhymenial layer, bends over and its two terminal nuclei undergo simultaneously a karyokinetic division in the prophase of which sixteen chromosomes may be counted. As first described by Dangeard, a terminal uninucleate and a penultimate binucleate cell are now cut off, the latter constituting the young ascus.

The terminal cell may continue its growth and give rise to a hypha, the penultimate cell of which again forms an ascus. The two nuclei of the ascus appear to be, in such cases, of the relationship of cousins.

Each of the two nuclei of the ascus now enters on the early prophases of hetero-

type division; the chromatin becomes aggregated to one side, constituting the 'first contraction figure' of Farmer and Moore. This is followed by the appearance of a more loosely coiled spireme in each nucleus, and, here and there, longitudinal fission of the thread may be seen.

At this stage the ascus-nuclei fuse.

The longitudinal split now becomes more evident, but later it disappears for a time as the fusion-nucleus passes into synapsis.

The subsequent stages of the first and second divisions in the ascus are in agreement with those described by Farmer and Moore for the spore-mother-cells of *Osmunda regalis*; as the nucleus passes out of synapsis, loops become obvious, each of which represents a bivalent chromosome. The chromosomes divide transversely in the first mitosis and, in the second, the longitudinal fission begun in the first prophase takes effect. In each of these divisions the number of chromosomes is sixteen.

This number was first observed, in *Humaria rutilans*, in 1904–5, by Guillermond, who also noted the occurrence of a longitudinally split spireme, and of a synapsis in the first division.

In the prophase of the third division sixteen bent chromosomes appear, but in the anaphases only *eight* chromosomes pass to each of the daughter-nuclei.

The spores are delimited by radiations passing out from the centrosome, but the direction of these is, to some extent, regulated by the position of neighbouring vacuoles, which may also aid in the delimitation of that part of the spore remote from the nuclear beak.

The processes observed in connexion with the development of the ascus suggest the following interpretation:—

The sporophytic number of chromosomes, as seen in the ascogenous hypha, is sixteen.

The ascus, when first formed, resembles other cells of the mycelium in being multinucleate[1]. Each of the nuclei, typically two in number, of the ascus enters independently on the meiotic phase. Fusion then takes place, the two spiremes becoming indistinguishably mingled.

The sixteen chromosomes which appear in the first and second divisions, and in the prophase of the third, may be regarded as representing two sets of post-meiotic (or gametophytic) chromosomes united within one membrane, half having been derived from each spireme.

On the spindle of the third division the chromosomes separate away from each other, and the true gametophytic number, eight, becomes apparent. The premeiotic (or sporophytic) number is restored by the apogamous fusions in the hypothecium.

It seems not impossible that the fusion of the nuclei in the young ascus is due to their close proximity at a time when the nuclear wall is disappearing. A probably similar fusion has been several times observed between two of the four nuclei present in the ascus after the second division. The fusion in the ascus would be thus in no sense sexual, having been, moreover, preceded by a sexual fusion, in this case reduced. It appears comparable rather to the fusions of sporophytic nuclei artificially induced by Němec in root-tips.

H. C. I. FRASER.

[1] Asci containing more than two nuclei were occasionally observed, but their fate could not be determined.

The Development of the Heterotypic Chromosomes in Pollen Mother-cells.

BY

DAVID M. MOTTIER,

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With Plates XXVII and XXVIII.

IN September, 1905, the writer (Mottier, '05) published a preliminary report on the development of the heterotypic or bivalent chromosomes in *Podophyllum*. The results of observations set forth in that communication were as follows: (1) That, in agreement with the conclusion of Farmer and Moore ('05) and others, the bivalent chromosome developed in the first or heterotypic mitosis is formed by the approximation of two chromosomes side by side, or otherwise, which were previously arranged end-to-end in the spirem, and not by the approximation of two spirems either before or during synapsis, as claimed by Grégoire and his students, Allen, Strasburger, and others. That the separation of these two chromosomes (i. e. the two segments, if the bivalent chromosome be considered as one) is a transverse division, and not a separation along the line of longitudinal fission observed in the spirem. (2) That there is no fusion of two distinct spirems as the nucleus goes into the first contraction or synapsis.

In the following communication, the writer hopes to present the results of detailed observations substantiating the above conclusions, and to show also that, in the plants considered, there is no definite or reliable evidence to support the pro-chromosome theory suggested by Rosenberg ('04), and especially emphasized by Overton ('05) for several dicotyledonous plants, and by Miyake ('05) for certain Monocotyledons. The entire mitotic history will be traced from the resting nucleus to the complete formation of the heterotype chromosomes in the pollen mother-cell of *Podophyllum peltatum*, *Lilium Martagon*, *L. candidum*, and *Tradescantia virginica*, although not in complete detail in the latter. A study of *Galtonia candicans* has also been made, and this plant was found to correspond in important details with *Tradescantia virginica*, as will be seen also from the results of other observers.

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METHODS.

All material was fixed in chrom-osmic-acetic acid, after the formula published by the writer in 1897. Both the triple and iron-haematoxylin stains were used. In so far as pollen mother-cells are concerned, the triple stain of anilin safranin, gentian violet, and orange G is superior when all stages of mitosis are concerned. The iron-haematoxylin is very valuable in the differentiation of the chromatin during the prophases. In preparations stained by the iron-haematoxylin, counter-staining with eosine, orange G, gentian violet, &c., was followed for a time, but as no special advantage could be observed in the use of the counter-stain, this part of the process was finally abandoned. Preparations of almost every stage of the process described in this paper have been made in duplicate, one-half of the slides stained with the triple, the other by the iron-haematoxylin. In this way one stain serves as a control for the other.

FROM THE RESTING NUCLEUS TO SYNAPSIS.

Podophyllum. The developing anthers of *Podophyllum* were examined during the autumn and winter in order to determine the nature of the chromatin in resting nuclei in the cells of both the sporogenous and purely vegetative tissues. In stamens fixed in early October, all stages of mitosis are to be seen in the cells that are to give rise to the pollen mother-cells, as well as in purely vegetative tissue. At this time of the year, the pollen mother-cells were not differentiated, at least, in all material examined. In stamens fixed January 20, the pollen mother-cells were still undifferentiated, and about all nuclei were in the resting condition. A careful study of material collected at the dates just mentioned and in the following spring did not reveal any disposition of the chromatin into sixteen masses or lumps that might be regarded as representing the somatic chromosomes. As will be shown below for the pollen mother-cells, the chromatin of the resting nuclei exists in masses or lumps of varying sizes, and connected into a network by slender threads composed chiefly of linin. The number of chromatic masses was always greater than the number of somatic chromosomes. In fact, it is not possible to determine the exact number of these lumps, for they vary greatly in size, and three or more masses are frequently so connected by thick strands that it is not possible to know how many lumps each of such masses really represents. Frequently, in both sporogenous and purely vegetative cells of an anther, the resting nuclei will present the chromatin in rather fine granules uniformly distributed within the nuclear network. This finely granular condition of the chromatin in resting nuclei is also met with in the pollen mother-cells. In all cells showing such a distribution of the chromatin, fixation seems perfect, and it

is impossible to say whether the breaking up of the chromatin into fine granules is a normal state or due to the action of fixing fluids.

Soon after the last division in the sporogenous tissue, which gives rise to the pollen mother-cells, the nuclei pass into the resting condition, and both cell and nucleus begin to increase in size, the nucleus enlarging at first somewhat faster than the cell. The nuclear frame-work consists now of numerous chromatin masses of varying sizes connected into a net-work by slender threads (Pl. XXVII, Fig. 1). These threads, which consist largely of linin, vary also in thickness. The thicker ones frequently retain the safranin more strongly, thus appearing to be somewhat in the nature of chromatin, and, under such conditions, a distinction between chromatin and linin seems very difficult to determine. One or more nucleoli are also present. Fig. 1 represents a rather thick section including a tangential view of the nucleus. Fig. 2 is a median section of a nucleus taken from the same anther. This figure shows that the chromatin has a peripheral arrangement in the nucleus, a condition which is frequently observed, and that the nucleolus lies in a cavity relatively free from the nuclear network. It is seen also that the chromatin masses are larger than in Fig. 1, and that their number is greater than sixteen; hence these lumps are not pro-chromosomes. Two masses are frequently observed lying more or less closely side by side, but there is no reason to believe that such pairs are fusing. The delicate threads joining the chromatin masses may be found lying close to each other and parallel, but this does not signify that a double spirem is in process of formation. Inasmuch as the nuclei in all pollen mother-cells in an anther loculus are in almost the same stage of development, it is not very probable that Figs. 1 and 2 represent very different stages, but rather the same or very closely related stages. The greatest difference observed in the stage of development of cells in different loculi of the same anther, before the formation of the hollow spirem, was found at the time when the synaptic ball begins to loosen up. In one loculus, the synaptic mass had just begun to loosen up, as shown in Fig. 6, whilst in a neighbouring loculus the spirem had loosened to the extent of almost filling the cavity of the nucleus; but the chromatin thread had scarcely become any thicker, nor had it assumed a very regular arrangement, being much entangled in places. If there be a difference in development between Fig. 1 and Fig. 4, Fig. 4 is certainly the older, i.e. further toward synapsis. In Figs. 3 and 4, we have nuclei that are frequently met with. In Fig. 4, the chromatin is in the form of small masses of a rather uniform size, and evenly distributed in the linin network. Each mass or granule is composed of very many fine granules. The nucleoli, as in such nuclei as Fig. 1 and Fig. 2, lie within spaces comparatively free from the nuclear framework. As pointed out by the writer (Mottier, '99) in a paper on the effect of centrifugal force upon the cell, the nucleoli seem to lie within a space that

does not contain any material capable of being stained by known methods. It may frequently happen that the nucleolus or nucleoli may lie in contact with portions of the chromatin or linin threads. In Fig. 3 the chromatin is aggregated into irregular, granular shreds, giving a picture strikingly different from that of Fig. 4. These two cells were drawn from the same loculus of the anther and from the same section. In a number of preparations, nuclei of cells in about one-half of the loculus presented the structure shown in Fig. 4, while in the other half, that in Figs. 1 and 3 prevailed, with all intergrading stages between. The cytoplasm in Fig. 4 is small-meshed and finely granular, giving a rather dense and finely granular appearance. In Fig. 3, a coarser and looser reticulum is present, and in Fig. 1 the fibrillar reticulum is still looser but very sharply defined and beautiful. There can be but little doubt that these nuclei are in about the same or in closely related resting stages; for all three conditions are found in the same loculus. In many preparations of the same stage of development of the anther, about all the nuclei possessed the type of structure shown in Fig. 1. This structure is more frequently met with in the developing sporogenous tissue of young anthers and also in purely vegetative cells, yet a more finely granular state of the chromatin is likewise to be found in vegetative cells. In the preparations from which these figures were drawn, there was nothing to indicate poor fixation; the cells seemed perfectly fixed and normal in every respect. There is, of course, a possibility that the difference here manifested in the disposition of the chromatin may be due to the effect of the fixing fluid, especially the part played by the osmic acid, but as to this the writer is not prepared to speak definitely at present. As will be shown later, it is after all a matter of comparative insignificance whether the chromatin occurs in larger or smaller masses or granules in the resting nucleus.

Overton does not find favourable evidence in behalf of the prochromosome theory in *Podophyllum*. He states ('05, p. 130) that 'in *Podophyllum peltatum* the prochromosomes are not recognizable as such. They may be represented by groups of chromatin masses which are connected by linin threads and arranged in parallel rows during the reticulation. These chromatin masses are distributed upon linin threads running parallel to each other, and, as in *Helleborus foetidus*, there are found two distinct and independent spirems, which pass into synapsis unpaired and unfused.' Overton supports this statement in his Figs. 53 and 54 (l. c., '05). In these figures are shown parallel linin threads upon which are arranged at rather long intervals pairs of chromatin masses. The nuclei from which such figures were drawn must occur very rarely, for among a large number of preparations of *Podophyllum* no nuclei were found with parallel linin threads arranged in such diagrammatic regularity. Overton either did not find or overlooked entirely such stages as figured in my Figs. 1, 2, 3, 4. In his Fig. 5, which

is stated to occur just before synapsis, a thicker chromatin thread is shown than in his Fig. 56, which is in synapsis. If Overton's Fig. 56 correctly represents the chromatin thread in synapsis, it seems to me that his Fig. 55 is a loosening up of the synaptic mass, and hence a later stage, for I have found no such condition as his Fig. 55 before synapsis.

Cardiff ('06) claims that, previous to synapsis in *Acer platanoides*, *Salomonia biflora*, *Ginkgo biloba*, and *Botrychium obliquum*, there are formed two or more chromatin threads which arrange themselves in pairs longitudinally and move together as synapsis approaches, finally fusing in synapsis. Judging from his own experience in the study of nuclear phenomena, the writer is of the opinion that Cardiff's figures are too diagrammatic to enlist confidence in the accuracy of his interpretation.

Both cell and nucleus increase rapidly in size, so that when synapsis is complete they have about reached their maximum dimensions. In *Podophyllum* the pollen mother-cells vary considerably in size, as do also the stamens in any given flower. In thicker and longer anthers larger cells are found, but whether this is always true no effort was made to determine. It is very frequently true, however, that the cells near the middle of the anther may be very much longer than broad. Fig. 6 represents a cell which is larger than the average.

Whether the chromatin undergoes any marked change from that shown in Figs. 1–4 before contraction, as will be pointed out below for *Lilium*, cannot be stated positively. With the rapid increase in size of the cell and nucleus, the entire contents contract or ball up toward one side of the nucleus. This contraction is a rapid process, and whether the rapid growth of the nucleus is a stimulus to this contraction is an interesting question. The nucleolus may be included partly or wholly within the contracted mass, or it may lie merely in contact with it, or entirely free and at a remote side of the nucleus. As a rule, the nucleolus is included within the chromatin mass (Fig. 5). When complete synapsis is reached, the mass is tightly balled up, many nuclei showing no free portion projecting into the nuclear cavity. Frequently a few shreds of linin, including some chromatin, protrude from the mass, and sometimes a small portion of the chromatin thread may project. In some cases very fine delicate threads extend from the mass across the nuclear cavity to the nuclear membrane. There seems to be little or no regularity in the position of the synaptic mass in the nucleus as regards the upper or lower ends or the sides. Cardiff states that in the plants studied by him gravity determines the position which the mass shall take. This explanation does not hold for *Podophyllum* or *Lilium*.

In the contracted mass no definite structure can be made out. Sometimes the appearance is that of a balled-up mass of lumps or granules, and sometimes that of a chromatin thread. The nuclear membrane in the

stage of synapsis does not present the sharp contour that is seen in the resting nucleus. On the contrary, it is very delicate and appears to be composed of a network of very delicate fibrils, or as a mere cytoplasmic boundary of the nuclear cavity. The nucleus must have, however, a distinct membrane of its own, for when one observes, for example, the male nucleus in the cytoplasm of the egg or in the cavity of the embryo-sac, one can scarcely conclude otherwise. Just how long the condition of synapsis persists cannot be stated definitely. The time undoubtedly varies with the rapidity of growth and may depend upon growth conditions. The writer has observed that pollen mother-cells of *Lilium* divide rapidly on bright warm days, while in cool cloudy weather the process went on much more slowly. The synaptic period represents probably the longest pause in the mitotic process, but the exact duration and the conditions regulating the same are matters yet to be determined.

As stated above, no definite structure can be made out with certainty in the mass when synapsis is complete, but as soon as the mass begins to loosen up, as is manifested by the extension into the nuclear cavity of, at first, a few, then several loops or turns of the spirem (Fig. 6), it is clearly seen that a definite spirem is present which is split lengthwise, as evidenced by the occasional divergence of the halves, or by the presence of two rows of chromatin granules or chromomeres. However, a double row of chromomeres cannot always be seen in the free portions of the spirem that first project from the contracted mass. Up to the time of synapsis, the pollen mother-cells are polygonal in form, fitting together in complete tissue connexion, but, as soon as the synaptic mass of the nucleus begins to loosen up, the cells round off at the corners, and in a short time they will have separated from the original walls and have formed the new walls so characteristic of these cells. As stated in my earlier publication, the original, delicate walls of the cells persist for some time after the pollen mother-cells have contracted away from them. The rounding up of the pollen mother-cells seems to be brought about both by the contraction of the protoplasm of the cells and by the enlargement of the anther through growth. With the further increase in size of the loculus, the cells soon come to lie loosely in the cavity. Before describing the phenomena immediately following synapsis and the final formation of the chromosomes, the presynaptic history in *Lilium* will be given.

Lilium. Shortly after the organization of the daughter nuclei, resulting from the last somatic division in the sporogenous tissue, the chromosomes begin to reticulate into smaller masses. One or several nucleoli may be present, usually more than one. The nucleus now presents a structure consisting of irregular lumps or masses of chromatin, varying in size and held together by linin threads or strands (Fig. 15). Many of these linin strands are thicker than at a later stage and retain the safranin strongly.

Because of this fact, these strands suggest the nature of chromatin. They do not, however, hold the stain as strongly as the chromatin masses, and there can be no doubt that we have a differentiation between chromatin and linin, although what the exact difference is cannot be stated. It has been observed in a number of preparations that, at the stage represented in Fig. 15, the chromatin masses show a tendency to group themselves closely about the nucleoli, and the linin strands to radiate from the collection. This condition gives a very noticeable appearance even with low powers of the microscope. What significance, if any, such a grouping may have, the writer is not prepared to say, neither is it known to be a regular and perfectly normal phenomenon; although the preparations show no evidence of abnormal fixing. In former publications the writer expressed the opinion that the nucleolus may contribute material for the formation of chromatin. By this he does not mean, as some observers have interpreted the statement, that the nucleolus is reserve chromatin, for he has no well-defined idea of what reserve chromatin may be, or that such a substance exists.

With further development of the anther, the pollen mother-cell and its nucleus increase in size. The chromatin masses increase through growth and become more regularly distributed within the nuclear cavity, but more especially along the periphery. The nucleoli lie within the nuclear cavity, which is, as a rule, freer from the network, and their connexion with the chromatin is less intimate, or they are entirely free (Figs. 16, 17). Here, as in *Podophyllum* the chromatic masses, which are always variable in size and in shape, are connected into a reticulum by means of linin threads. These threads are sometimes slender, showing very fine granules; sometimes they are thicker and retain to some extent the chromatin stain. The same phenomena are manifested, whether the stain used was the triple or the iron-haematoxylin; but for the differentiation into chromatin and linin the triple stain seems to be superior. It may be remarked also that the number of these masses always far exceeds the somatic number of chromosomes. In preparations of this and later stages (Figs. 15, 16, 17) which are not too densely stained, it is to be seen that each chromatin mass is composed of many smaller granules (Fig. 18). Fig. 18 was taken from the same anther loculus as Fig. 16. In this the stain differentiated in such a manner as to leave the linin very much paler or almost colourless. A similar condition is figured by Allen for *Lilium canadensis*. In the period of development in *L. Martagon* between the stage shown in Figs. 16 and 17 and synapsis, there seems to be undoubtedly a very remarkable and striking variation. After a slight increase in size, nuclei, presenting the structure of Fig. 17, may undergo the synaptic contraction (Figs. 22, 23). On the other hand, the chromatin masses may undergo a further reticulation into finer granules, so that the nucleus reveals a linin

thread, or network, with a single row of chromatin granules (Fig. 19). In some preparations, nuclei with the structure indicated in Fig. 17, occupy one end of the loculus, while in the other end of the same loculus the structure of Fig. 20 is present, and in the middle portion complete synapsis. In other preparations, nearly all the nuclei in the pollen mother-cells of the whole anther show the structure of Figs. 19, 20, 21, i.e. from a spirem or network with a single row of small granules to complete synapsis. In one end of such an anther there may be a few cells with the structure of Fig. 17; in others there are no such nuclei, but all show the structure as described in the preceding sentence. In none of the preparations was there anything to indicate unusual or faulty fixation.

Examining each of these conditions now in detail, it is seen that in Fig. 22, the chromatin masses are collecting into a mass at one side of the nucleus, as all conceivable stages between Figs. 17 and 23 are present in the same loculus. In Fig. 22, the linin threads are very beautifully shown, some being very delicate and staining blue or appearing colourless, whilst others are thicker and hold some of the safranin stain. Here and there two or more of these linin threads may have a parallel course, and some of the chromatin masses are paired, but how such phenomena can justify the doctrine that a double spirem is being formed is difficult to comprehend. There seems to be as strong evidence that a triple or quadruple spirem is formed, because the chromatin masses are frequently aggregated in clusters of three or more. In the second condition described above, we have a structure just preceding synapsis which differs from that shown in Fig. 22. Here it is certain that the chromatin becomes more finely reticulated, so that small and uniform granules are formed, and that these granules are being arranged into a single spirem (Figs. 19, 20). In nuclei presenting the structure of these figures, the nucleolus, or nucleolar material, is almost always flattened in one or two masses against the nuclear membrane. The chromatin thread is very frequently oriented with reference to the flattened nucleolus, as indicated in Fig. 20. This orientation gives the impression that the thread is disposed in many interlacing loops or turns, that extend out from the flattened nucleolus. There seems to be no closer connexion, however, between nucleolus and chromatin thread in this condition than in the other. Some preparations show that in the entire anther all pollen mother-cells were in some stage between that of Figs. 19 and 21. In Fig. 21 the synaptic condition is about complete. In the section from which this figure was drawn, about one-third of the nuclei presented the contracted mass, while in the rest some such condition as Figs. 19 and 20 was present. In such cells it was noticed that the flattened nucleolus was very constant and thereby conspicuous; but this was less so in the former condition, although it is frequently met with (Fig. 23). In preparations showing the structure of

Figs. 19 and 20 that were stained with the triple stain, the linin portion is colourless, while the chromatin granules remain blue. This means, of course, that in the process of differential staining the safranin is removed from the linin and chromomeres almost completely, and that the latter retain the violet stain with greater avidity. The condition of the nucleus as shown in Fig. 19 has been described by Farmer and Moore ('05, p. 508) for they state that 'the definite spirem thread can be distinguished very clearly at an early period in karyokinetic activity, certainly long before the spore mother-cells dissolve their union with each other. It forms a colourless thread, at first infiltrated with chromatin throughout, but the latter soon collects into serial beads so as to give rise to the well-known alternation of stainable (chromatin) and non-staining (linin) disks.'

Farmer and Shove ('05) figure a similar presynaptic structure in *Tradescantia virginica*. A spirem of a single row of granules has been described by Schaffner ('97, '06) as representing the presynaptic condition in *Lilium philadelphicum* and *L. tigrinum*. In fact this has been the interpretation of most cytologists in former years. Allen ('05), on the contrary, does not mention any such stage as the writer's Fig. 19, as occurring prior to synapsis.

From what has been described in preceding paragraphs, the possibility is not excluded that the chromatin may not always be in the same state of reticulation, or distribution within the linin framework, prior to the contraction into the dense mass. In Figs. 22 and 23, which do not seem in any way to represent artefacts, the chromatin exists in the form of lumps or masses, while in other cases (Figs. 19, 20, 21), there has taken place a much finer reticulation of the chromatin, so that the granules are much more numerous, of uniform size, and arranged in a single row within the linin reticulum. Whether we have in Figs. 19 and 20 a continuous thread, or thread and network combined, cannot be stated with absolute surety; but it is certain that a large part of the structure is a thread (Fig. 20) with only one row of granules, or we may say chromomeres. When we compare Fig. 18 with Fig. 19, it seems reasonable that the structure of Fig. 19 has been derived directly from that in Fig. 18. Anthers, showing the structure of Figs. 19, 20, 21, seemed to be in about the same stage of growth as those in which Figs. 22 and 23 were found, especially when one remembers that Figs. 16 and 18 were drawn from the same section. In all cases the pollen mother-cells, up to the time of synapsis, are contiguous throughout, forming a continuous tissue connexion without any intercellular spaces at the corners of the cells.

Whether the disposition of the chromatin, as described and figured for the plants in question, is due in any respect to the action of fixing fluids or not—this seems certain, namely, that, before or during synapsis, the chromatin becomes resolved into relatively small granules which do not

vary greatly in size, and which are arranged into a spirem. When such conditions as Figs. 19, 20, and 21 are found in preparations showing no evidence of poor fixation, the only conclusion that can be drawn is that the presynaptic thread consists of a single row of chromomeres. There is no evidence that two distinct spirems are present prior to the first contraction, or synapsis.

From Synapsis to the Loose or Hollow Spirem.

Podophyllum. As stated in the foregoing, the period of synapsis represents probably the longest pause in the entire mitotic process. During the same, the cells and their nuclei may increase somewhat in size, although extensive measurements were not made to determine the amount, as this was not considered of very great importance.

The first indication of a loosening of the contracted mass is seen in the extension into the nuclear cavity of loops of the spirem, and the beginning of the rounding off of the pollen mother-cells at the corners (Fig. 6). In the section from which this figure was taken, some of the cells showed the first signs of rounding up. When the loosening of the spirem has once begun, the process seems relatively rapid, as the enlargement of the anther is also rapid, so that the pollen mother-cells are soon separated from each other, in some instances rather widely. As soon as the balled-up thread begins to loosen, some of the loops or portions of the thread which project into the nuclear cavity are seen to be double, that is, two rows of granules or chromomeres may be made out in them (Fig. 6). The double row of chromomeres is not everywhere distinguishable, for in many of such free portions of the spirem only a single row can be recognized. In exceptional cases the halves of the thread diverge for a short distance, so that it seems that the linin thread is also double, but, as a rule, the linin seems undivided. The writer wishes to emphasize the fact that neither in *Podophyllum* nor in the other plants to be described further on, are the chromomeres all of the same size, nor are they all exactly paired (Fig. 6). In a comparatively short time, the contracted mass distributes itself somewhat uniformly within the nuclear cavity, and the pollen mother-cells round off, secreting their own rather thick and characteristic cell-walls. This stage is shown in Fig. 7. In nuclei less densely stained, the two rows of chromomeres imbedded or held in the linin can be clearly observed. In exceptional cases the halves of the spirem may diverge in places, showing clearly the longitudinal fission of the spirem. In *Podophyllum*, the separation of the halves is the exception (Fig. 7). Following this stage, the spirem thickens somewhat and probably becomes a little shorter, but the contraction or shortening is very much less than has hitherto been assumed. The result is a smooth chromatin spirem, arranged in rather regular turns, and in which, as a rule, no indication of a longitudinal fission

is to be recognized with any certainty save in exceptional cases. The regularity of the hollow spirem is more pronounced in *Podophyllum* than in many other plants observed by the writer, but the regularity is not so great as in those plants in which a diagrammatic arrangement of the thread into pole and anti-pole sides has been described. The stage of the regular, hollow spirem is followed by a tendency of portions of the chromatin thread to become arranged in long loops (Fig. 8) preparatory to passing into the second contraction, for a second so-called contraction is as certain in *Podophyllum* and in *Lilium* as there is a synapsis, although, as will be shown, this contraction is different in character and duration. The important thing to be borne in mind as regards *Podophyllum* is that, in the stage of the hollow spirem, we have a smooth and rather regularly arranged chromatin thread, in which all traces of a longitudinal split are obliterated save in exceptional cases, and consequently the sister halves of this thread do not diverge, as frequently happens in *Lilium candidum.*

In *Lilium.* Turning now to *Lilium Martagon* and *L. candidum*, it will be observed that a similar sequence of events obtains here as in *Podophyllum.* Synapsis leaves the nuclear contents tightly massed or contracted into a ball, which, as a rule, lies close to the nuclear membrane at one side. In some instances the mass seemed to be more central in position. I was unable to determine any regularity in the location of the mass that might lead to the conclusion that the side against which it lay was determined by gravity. Very frequently, delicate colourless fibres or threads extend from the contracted mass to the nuclear membrane (Fig. 24). Here also the loosening of the mass is first recognized by the extention of loops or turns of the spirem (Fig. 24). As in *Podophyllum*, the chromatin thread is seen to be double. This is especially apparent in cases where the halves of the spirem diverge for a short distance (Fig. 24, at the left). The loosening continues until the hollow spirem is reached (Figs. 25, 26). The turns of the spirem, though loosely arranged, are not disposed in any marked diagrammatic regularity. There are long and regular turns intermingled with short and abrupt curves and kinks. The spirem traverses the cavity of the nucleus as well as to follow its membrane. At this stage (Figs. 25, 26), usually one large nucleolus is present, but one large and one or more smaller nucleoli may occur. In *Lilium* the chromatin spirem is very long and slender at the stage in question. In sections including an entire nucleus, it is not possible to follow the thread accurately throughout its entire course. Some parts will always be concealed by others, and for that reason I have made no attempt to represent the impossible in a camera lucida drawing. Fig. 26 represents the larger part, but not the whole nucleus. It cannot be stated, therefore, whether free ends are present, but it is the opinion of the writer that the thread is endless, although this may be, and doubtless is, a matter of no importance.

In anthers, containing the stage of Figs. 25 and 26, some of the pollen mother-cells fix and stain in such a manner that the chromatin and linin make-up of the thread is very clearly shown. For example, it is found that in one end of the anther, the cells will so differentiate in staining with either the triple or the iron-haematoxylin method, that the chromomeres are brought out very distinctly, while in the remaining part of the anther the stain is held more uniformly by the thread, and the differentiation into linin and chromomeres is not so apparent. Figs. 25 and 26 may be found in the same section of a loculus. In Fig. 25, the safranin washed out of the thread almost completely, and, as a result after staining with gentian violet, the linin remained colourless or nearly so, and the chromomeres blue. In Fig. 26, which is typical of all remaining nuclei in the loculus, the spirem retained the safranin more uniformly throughout, and the result was a more uniform thread, showing, however, the longitudinal fission, and the chromomeres in certain parts as a series of small lumps. As stated, the same differentiation is obtained by the use of the iron-haematoxylin stain. Whether these nuclei are to be regarded as slightly different stages, or as representing conditions in which the fixing fluid preserved the structures in such a manner as to render chromatin and linin capable of retaining the stains in a different degree, is a question. It not infrequently happens that, in a number of cells in one end of the loculus, the chromatin will differentiate as shown in Fig. 25, but in all other cells of the same loculus the condition of Fig. 26 prevails. However, as we know that nuclei in the same loculus are frequently in different mitotic stages, the fact that some of the nuclei in the end of an anther differentiate as in Fig. 25, and others as Fig. 26, may be due to differences in the stage of mitotic development. In the cytoplasm of these cells there is also some difference in appearance. The cells showing the nuclear structure of Fig. 25 have apparently a more finely granular cytoplasm, in which the fibrillar nature is not as clearly brought out as in those cells with nuclei of the structure shown in Fig. 26. In neither case, however, was there anything that could be looked upon as bad fixing.

So far as known to the writer, the true nature of the chromatin thread immediately following synapsis can be more clearly demonstrated in *Lilium* than in any other plant. Here it is shown with sharply defined clearness that the spirem consists of what is almost universally known as linin, in which are held the granules equally well known as chromatin. The chromatin granules are arranged, for the most part, in two rows, which, the writer believes, is the result of a true longitudinal fission. Each granule is usually spoken of as a chromatin disk or chromomere. It is also evident that each chromomere is composed of a number of smaller granules. According to the writer's interpretation, each chromomere is not in the form of a disk, and he desires to emphasize again, as he stated a number of years ago, that the spirem does not consist of alternate disks

of chromatin and linin, but that the chromomeres are held in the linin. Each chromomere has a rough outline, as if erosed, which seems to indicate that each is made up of smaller granules. The chromomeres were not observed in the form of angular blocks or masses, as figured by Allen ('05). In some parts of the thread the chromomeres are seen to be regularly paired and of the same size; but in other parts they may be different in size, some being much smaller than the average, and not paired but arranged alternately. Whether this is due merely to the twisting of the spirem cannot be stated with certainty at present. Fig. 25*a* represents a portion of the spirem at the stage of development in question, as the writer has observed it.

After the stage of Fig. 26, the spirem becomes slightly thicker, and always stains as a more uniform thread in which the double nature cannot always be made out; but frequently the double character of the thread is apparent, and it is seen that the two segments are twisted about each other (Fig. 26). In preparations of this stage of mitosis, cases in which the longitudinally split spirem tends to diverge are the exception rather than the rule (Fig. 26). The rule seems to be that the halves of the spirem are so closely applied to each other as to conceal nearly, or quite, the double nature.

From the stages shown in Figs. 25 and 26, the spirem soon passes into the condition which has been described as the second contraction (Figs. 27, 28). In these figures it is apparent that the larger part of the spirem is arranged in loops which tend to radiate from the centre of the nucleus, where the thread is already somewhat closely entangled. In this stage, however, as will be mentioned in a subsequent paragraph, the sister threads do diverge considerably in many instances.

When the chromatin thread, as it emerges from the synaptic mass, is compared with the presynaptic phenomena, certain changes seem to have occurred in the interval, although it is not possible to determine even in very thin sections what actually takes place in the dense contracted mass. The writer assumes that all nuclei pass through this stage, and he has limited the term synapsis to this contraction. Judging from figures of other observers, this term seems to have been made to include the loosening up of the spirem, and thin tangential sections of the early stages of the loosening mass have been doubtless looked upon as indicating what occurs in the tightly balled-up nuclear contents. Now when we compare the chromomeres in the spirem, as it emerges from synapsis (Figs. 6, 24), with the chromatin masses, as they appear in certain cases at the beginning of the contraction (Fig. 22), it is clear that these masses of chromatin are very much larger than the chromomeres. There must take place then, in cases like Fig. 22, a further reticulation into smaller granules. In such cases there would be as good ground for the conclusion that a double

spirem is formed in the synaptic mass as for the doctrine that a single spirem is formed, which then underwent a longitudinal fission. On the other hand, when we consider the condition of the chromatin shown in Figs. 19, 20, and 21, it is clear that a single spirem, with a single row of chromatin granules, is present just prior to synapsis. There is no evidence that, in such a nucleus as Fig. 19, two spirems are present. The chromomeres in this case are smaller than those in the post-synaptic spirem, and this fact finds its most reasonable explanation in the assumption that the chromomeres of the stage of Fig. 19 unite to make the larger chromomeres which split longitudinally to form the double row in the spirem. The doctrine that the longitudinally split spirem is formed by the union side by side of two distinct spirems rests purely upon theoretical considerations, and furthermore, when we know, as will be shown in what follows, that the two members (chromosomes) forming the bivalent chromosomes are arranged end to end in the spirem, all theoretical support for the assumption of the lateral fusion of two spirems will have fallen to the ground. The explanation remaining, which is most in harmony with all known facts, therefore, is that the longitudinal fission in the heterotype mitosis is as real as in any somatic division.

The Formation of the Bivalent Chromosomes from the Loose or Hollow Spirem.

Podophyllum. As stated in a preceding paragraph, the longitudinally split spirem, on coming out of synapsis, thickens and shortens to some extent, as it takes on the form of a rather loose and regularly arranged chromatin thread. With this process almost every trace of the double nature disappears, so that, in the absence of any knowledge of a previous fission, it would not be possible to say that the spirem is double, since the halves are so closely applied as to be indistinguishable (Fig. 8). In cases in which the double nature can be seen, the segments are usually twisted about each other, as described by the writer in 1897. Between the stage of Fig. 8 and the cross segmentation into chromosomes, the spirem undergoes comparatively little shortening. It has been assumed by earlier observers that the spirem shortens greatly, becoming very much thicker, and that the sister threads diverge widely in many places, before the cross segmentation takes place; and such an assumption is necessary under the explanation that the halves of the bivalent chromosomes lie side by side lengthwise in the spirem. Such, however, is not the case. The spirem now tends to become arranged in the form of loops (Fig. 8). This looping leads to the second contraction, by which the loops and other parts of the spirem that are not looped contract toward the centre of the nucleus in such a manner that the loops, the parallel sides of which have become more or less closely applied and twisted about each other, tend to radiate from the more

central and entangled mass (Figs. 9, 10). During this second contraction or rearrangement the spirem becomes partly or wholly segmented into the chromosomes. In some cases the twisted loops radiate somewhat diagrammatically, but this regularity is only exceptional. Fig. 9 and 10 which are slightly different stages, show average conditions met with in the many preparations. In Fig. 9 the spirem has not completely segmented, but in Fig. 10 segmentation seems to be complete or nearly so. In this and in nearly all other cases the entangled state of the chromosomes obscures their inner ends. In a great many instances the loops and other parts of the spirem are more entangled than is shown in these figures. From Figs. 8, 9, and 10 it is seen that the two segments, which are more or less twisted about each other, represent the bivalent chromosomes, and that each bivalent chromosome is formed by the coming together or approximation of two portions that were previously arranged end to end in the spirem. In *Podophyllum* there is no marked divergence of the longitudinal halves of the spirem during rearrangement, as may occur in *Lilium*. In the majority of cases this approximation of different parts of the spirem side by side is accomplished by a looping, but the segments may come together side by side, or adhere end to end after cross segmentation, as will be seen especially in *Tradescantia*. Frequently the curved ends of the radiating loops are directed toward the nuclear membrane with the free ends at the centre, but this is not always the case, and it may not be the rule, for the free ends are often seen pointing towards the periphery (Fig. 10). The free ends of the chromosomes are almost invariably connected with the nuclear membrane by delicate fibres. It sometimes happens that the longitudinal split, which is seen in the spirem on coming out of synapsis and which disappears from sight in the hollow spirem, can be observed in each parallel part of a loop (Fig. 10). The writer understands that this longitudinal fission of the spirem persists, but from the stage of the hollow spirem to the meta- or anaphase the halves are so closely applied as to obscure the split. During the anaphase, or in the early telophase, the longitudinal fission again becomes evident, and represents the line of separation of the daughter segments in the second, or homotype mitosis. In my earlier paper this fission was regarded as a second longitudinal splitting (Mottier, '03).

Between the time of the segmentation of the spirem into chromosomes, which takes place either during or following the second contraction, and the formation of the spindle, they shorten rapidly and become very much thicker. This, and not during the hollow spirem stage, it may be added, is the period of greatest shortening and thickening of the chromosomes in all plants observed. An error made by the writer in common with almost all other observers was the assumption that the greatest shortening and thickening of the spirem took place before cross segmentation.

Soon after the stage shown in Fig. 10, the chromosomes separate and become more or less scattered within the cavity of the nucleus. The segments of the chromosomes (i. e. the two chromosomes of the bivalent structure) are variously oriented toward each other. They may still adhere as a loop, as an open or closed ring or link, as straight thick rods lying closely side by side, or attached end to end. They may or may not be twisted upon each other. These and various other dispositions of the segments toward each other may be observed in all stages from the time of complete cross segmentation of the spirem to the mature spindle (Figs. 11, 12, 13, 14). In Fig. 14 are shown a few of the various forms of chromosomes observed in the spindle. Of these, that of Fig. 14 *a* is less frequently observed. The arrangement of the two segments toward each other doubtless depends on the manner in which the spirem was looped or entangled at the time of cross segmentation. So far as the writer can see, no special significance can be attached to the various orientations of the two segments toward each other.

Now, when we take into account the development of the chromosomes of the heterotypic mitosis from the hollow spirem, but one conclusion seems possible, namely, that each chromosome appearing in the equatorial plate of the spindle is bivalent, consisting of two chromosomes that were disposed end to end in the spirem; that the two segments of the whole chromosome are not daughter segments but two somatic chromosomes. From what is known during the anaphase, it is clear that each of these segments, or somatic chromosomes, is in turn double, consisting of two daughter segments which arose by the longitudinal splitting seen immediately following synapsis. Each bivalent chromosome may, therefore, consist of four chromosomes, each member of the bivalent chromosome consisting of two daughter segments. Consequently the first mitosis is a reducing division, that is, the numerical reduction of the number to one-half is accomplished, one-half of the somatic chromosomes going to one daughter cell and the other half to the other.

Lilium. In the period of the prophase of *Lilium*, between that following synapsis and the transverse segmentation of the spirem, certain details are more complicated and may lead more readily to different interpretations. For example, in two closely related stages, it is frequently difficult to determine which is younger or older, and a careful study and comparison of the figures of the several observers seem to indicate that older stages in the prophase have been interpreted as representing earlier steps in the process. Such errors, although slight, are sometimes sufficient to throw the observer's judgement against the conclusion that all other facts seem to support.

As pointed out in a preceding paragraph, the loose spirem, following synapsis, and which is longitudinally split, soon reaches its stage of greatest

regularity (Fig. 26). It should be borne in mind that in *Lilium* there is no diagrammatic regularity in the arrangement of the spirem throughout its entire course; some of its turns follow the nuclear periphery, while others traverse the interior. In the interior of the nucleus it is not possible to follow accurately the entire thread, but it seems that there are no free ends. In this stage, as in *Podophyllum*, very frequently no definite trace of a longitudinal splitting is discernible. The stage of the loose and more regular spirem soon undergoes a rearrangement and passes into that of the second contraction, which is first indicated by a conspicuous knotting or entangling of the spirem, usually near the centre of the nuclear cavity, and by a tendency of the thread to form loops, some of which radiate from the entangled centre (Fig. 27). At this stage of development, there is a marked tendency of the sister halves of the longitudinally split spirem to diverge rather widely in longer or shorter stretches of the thread, and, in the opinion of the writer, this fact more than any other has led to a misinterpretation of the stages which follow. A careful study of the preparations showing this and closely related stages shows that, while the divergence of the sister halves is frequent at this stage, it does not seem to be the rule; for cells in which little or no divergence of the longitudinally split halves are found side by side with those in which the divarication is greatest. It does not seem probable, therefore, that the wide divergence of the halves is a rule, or that it represents a stage through which all nuclei pass. However, as will be seen, the halves come together again at a later stage and become so closely applied that no trace of the longitudinal fission can be clearly made out.

The further advance of this rearrangement, or second contraction, is manifested by a larger central entanglement of the spirem in which the nucleolus may be included (Fig. 28). A large part of the spirem is arranged in loops that tend to radiate from the centre, with the head or bend of many loops toward the nuclear membrane. All the spirem, however, is not disposed in the form of loops, but some parts may have a straight course, while others consist of short and irregular turns. It will be seen also that the free ends of some of the loops will be turned toward the periphery, and that the number of loops does not necessarily correspond to the number of chromosomes. In Fig. 28 the longitudinal fission can be seen in certain of the loops, but the halves do not diverge, while in other parts of the spirem all trace of this fission is obliterated.

The stages occurring between Fig. 27 and the complete differentiation of the chromosomes are those that offered the greatest difficulty to those who have worked with this problem, and, as stated in a preliminary communication of the writer[1], they represent those steps that were regarded as the product of poor fixation at the time of his earlier study. There is now,

[1] Bot. Gaz., loc. cit., xl: '05.

however, no very good reason to believe that these steps represent other than normal stages through which all nuclei of the pollen mother-cells of *Lilium* pass, as well as in many other plants. At the stage of Fig. 28, the double spirem is slender, having shortened and thickened very little, for the period of greatest shortening and thickening begins with, or after, the cross segmentation. The processes following the condition of the spirem shown in Fig. 28 result in that illustrated by Figs. 29, 30, 31. From these figures it is clear that adjacent and parallel portions of loops approximate side by side and in many cases twist about each other. The degree of approximation and twisting of portions of the spirem always varies in the same nucleus. Also the regularity in the arrangement of the loops and other parts of the spirem varies greatly in different nuclei. Diagrammatic regularity is the great exception. The figures commonly seen are shown in Figs. 29, 30, 31. The most regular cases were not selected for illustrations, and in the figures cited the writer has endeavoured to represent average conditions observed in the majority of nuclei. In Fig. 29, for example, the looping is much less regular; three or more smaller nucleoli are present, and a larger part of the thread is knotted and entangled in the central mass. At this stage it is not possible to say that the cross segmentation is complete, but it is certain that some of the chromosomes are differentiated (Figs. 30, 31). In the light of these facts, it is scarcely possible to conclude that parallel parts of the loops, which are more or less twisted about each other, represent the halves of the spirem brought about by the longitudinal fission. In some cases it is to be seen that each parallel part of the loop is double, such a double nature being due to the longitudinal fission (Fig. 30). When the stage in the prophase represented by Figs. 30 and 31 is reached, it is probable that cross segmentation is almost completed. Some of the chromosomes, perhaps the majority that are loops, have the bend of the loop directed toward the nuclear periphery, but others are oriented with the free ends thus situated. All of the chromosomes are not formed as loops; many appear as two rather straight or bent pieces free at both ends, which have come to lie side by side (Figs. 31, 32). Whether the approximation in these cases came about before complete cross segmentation cannot be stated. In the stage shown in Figs. 30 and 31, the chromosomes are connected with each other and with the nuclear membrane by very delicate linin fibres. It is true, of course, that the chromatin is always in communication with the nuclear membrane and cytoplasm by means of such fibres.

Following the stages of Figs. 30 and 31, the chromosomes separate from each other and tend to distribute themselves within the cavity of the nucleus. At the same time each contracts rapidly, becoming thereby shorter and thicker, and the fact that the two pieces of each chromosome are split longitudinally can no longer be definitely recognized, although

this can be seen in exceptional cases. The shortening of the chromosomes continues, and they are soon more regularly spaced within the cavity (Fig. 33). There is no great regularity in the spacing; sometimes they seem to be quite regularly disposed, but frequently two or more lie close together or in contact. Fig. 33 represents the nucleus just prior to the formation of the multipolar spindle complex, and the chromosomes have about the form that they will present in the equatorial plate apart from a further shortening. The chromosomes show clearly that some arose as loops, while others may have come about by the approximation of two pieces which may, or may not, have been entirely separated before the approximation. The segments are generally twisted about each other, although this is not universally true. It is not necessary to mention the various shapes of the chromosomes as found after their formation from the spirem and upon the nuclear spindle, since the various forms arising from the manner in which the two pieces are oriented with respect to each other have already been figured in superfluous profusion by other observers. It may be stated, however, that the number of phantastically-shaped chromosomes will depend upon the degree in which the spirem is entangled, knotted, and kinked during the second contraction and earlier. It is reasonable to believe that chromosomes coming from such nuclei as Figs. 29 and 31 will be less regular in form than those arising out of those similar to Fig. 30.

When the formation of the loops during the second contraction is duly considered, and when it is recalled that the chromatin thread shortens comparatively little in the stage of the hollow spirem, and that the sister halves of the thread, if divergent, become again closely applied,—only one explanation seems now possible to the writer, namely, that the twisted parallel parts of the loops shown in Fig. 30 result first from a looping of the spirem, followed by a more intimate approximation of the parallel sides of the loop. The somatic chromosomes are, therefore, arranged end-to-end in the spirem and not parallel side by side. There seems to be no good ground for believing that the longitudinal halves of the spirem tend always to diverge widely for longer or shorter stretches and to remain thus separated; that the divergence is followed by a sudden shortening and cross segmentation into chromosomes; and that the daughter segments fuse at one end following cross segmentation to form the loops so frequently and so abundantly present. Such a process, or one similar, must be assumed by those who believe in the doctrine that the longitudinal fission is the lateral union of two spirems in synapsis, and that the two segments of each chromosome appearing in the equatorial plate of the heterotypic spindle separate along the line of longitudinal fission.

Tradescantia. The development of the heterotype chromosomes in *Tradescantia virginica* agrees in general with that described in the pre-

ceding pages. Because of this fact only those steps will be described and illustrated which seem to the writer to be necessary to clear up certain details upon which the main difference of opinion rests.

The nucleus of the pollen mother-cell in *Tradescantia* just prior to synapsis is shown in Fig. 34. Here it is seen that the chromatin is distributed in the linin network in the form of lumps or granules of rather uniform size, which show a tendency to aggregate into masses or groups. Miyake ('05) has evidently represented a similar condition in his Fig. 135. Judging from the series of figures of Farmer and Shove ('05, Figs. 23–26), it would seem that the chromatin granules are more finely divided, unless the difference between their illustrations and that of the writer is due to a difference in magnification or in methods. Whether the chromatin becomes more finely granular before synapsis, as shown for certain cases in *Podophyllum* and *Lilium Martagon*, cannot be stated. However, there is no doubt but that in some cases, at least, the structure of Fig. 34 passes directly into synapsis, for, in some preparations, the nuclei in one loculus were found with this structure, whilst in the adjacent loculus nearly all nuclei showed almost the completely balled-up condition. Inasmuch as the nuclei in the same anther are nearly all in the same or very closely related stages, there can be little room for doubt concerning the accuracy of this statement. Farmer and Shove neither describe nor figure the tightly balled-up condition of complete synapsis. They represent the first contraction figure as a partly contracted spirem only (l. c., Figs. 27, 28, 29) without an evident nuclear membrane. In all preparations of the writer showing this stage, a well-defined boundary always exists between the nuclear cavity and cytoplasm. The pollen mother-cells still form a compact tissue, but before the contracted ball has progressed far in its loosening up the cells round off and separate. It seems that the rounding off of the cells occurs sooner, or perhaps more rapidly, than in the other plants here described.

As the spirem emerges from the contracted mass, it is seen to be double or split lengthwise, though the halves divaricate only slightly at certain places. On coming out of synapsis there is in many nuclei a very marked regularity in the loops or turns of the spirem as they emerge from the remaining entanglement, which suggests the 'bouquet stage' described for certain insects (Fig. 35). According to Farmer and Shove (l. c., Fig. 32), the chromatin thread of the hollow spirem is disposed in the form of very uniform loops, showing a marked pole and anti-pole sides. Miyake's figure (l. c., Fig. 138), however, does not show any greater regularity than in other plants.

The rearrangement of the hollow spirem, or the so-called second contraction, agrees in general with what has already been described in the preceding pages. The regularity in the formation of the loops is somewhat

less marked, and they are fewer than in *Lilium candidum* (Figs. 36, 37). The spirem segments in part, at least, during the second contraction. The loops and the parallel straight or curved pieces of the thread often show a marked tendency to radiate from the entangled remainder (Fig. 37). This occurs simultaneously in a large majority of the nuclei in a given loculus, so that the picture obtained, even with low powers, is sufficiently characteristic to attract attention at a glance. When in the stage of Figs. 36 and 37, which seems to be a regularly occurring step in this mitosis, the longitudinal fission, observable always at an earlier stage, cannot be recognized except in rare cases, and there can be no doubt but the loops and parallel pieces are due to an approximation of different parts of the spirem. This interpretation is borne out as the entangled mass of Fig. 37 is loosened sufficiently to show the individual chromosomes in their entirety. There is, however, a marked peculiarity shown in *Tradescantia*, which has been noted also by Miyake for this plant and by Strasburger for *Galtonia*. As soon as the chromosomes become more evenly distributed in the nuclear cavity, following the second contraction, some appear in the form of loops with closely applied sides; others consist of paired rods, either straight or variously bent, and still others are connected into a chain, giving the impression that the spirem had not completely segmented, or that certain segments had become attached end-to-end after cross segmentation (Figs. 38, 39). This end-to-end attachment of some of the chromosomes persists in some cases until metakinesis. In the stages of Figs. 38 and 39 and during the formation of the spindle (Fig. 40), the two members of the chromosomes may form short loops, with parallel parts more or less closely applied and either straight or twisted; or the loops may be contracted into thick rings or links, open or almost entirely closed, or the two segments may adhere endwise.

When the formation of these chromosomes is followed in detail, and when their structure is observed in the spindle during the anaphase (see Figs. 30–33, published by the writer in Bot. Gaz., xxxv, 1903), it is plainly evident that each is bivalent and that the two segments are really two chromosomes that were arranged end-to-end in the spirem. When we recall also that during the anaphase (Mottier, '03, Fig. 33) each of these segments is split lengthwise, it is highly probable that this is the fission observed in the spirem as it emerges from synapsis.

Galtonia. A thorough study of all stages of *Galtonia* was not made, but so far as observation extended the process was found to agree more closely with that of *Tradescantia* and *Podophyllum* than with *Lilium*. This fact is now apparent from the figures of Strasburger ('04) and Miyake ('05). The interpretation of the formation of the chromosomes in this plant, published by Strasburger in the Sitzungsberichte der Königlich Preussischen Akademie der Wissenschaften, is, therefore, the correct one.

The behaviour of the chromosomes from their appearance in the nuclear plate of the first mitosis to the end of the second or homotype division has been described by the writer in an earlier paper (Mottier, '03), and, as a re-examination of that part of the mitotic history has only confirmed my former observations, a repetition seems superfluous here. It is to be noted, however, that the longitudinal fission of the chromosomes during the anaphase or telophase, which was then regarded as a second longitudinal fission, is, of course, the first and only such fission in these mitoses.

General Considerations.

A consideration of the important facts brought out in the preceding pages seems to point with a high degree of probability to certain definite conclusions, and to indicate more or less definitely several lines of study that may lead to fruitful results. In looking over the literature of the past ten or twelve years, which deals with the mitotic process in reproductive cells among plants, one is impressed with the fact that, at different times, the attention of observers has been focused especially upon some certain phase of the karyokinetic development. As in most problems, presenting many and great difficulties, it has been possible to clear up but one point at a time. For a certain period the chief interest centred about the centrosome and the development of the spindle, with the behaviour of the chromatin taking second place. At present, however, the question of centrosome and spindle development may be regarded as pretty definitely settled, especially among the higher plants, while concerning the behaviour of the chromatin there is still much diversity of opinion among both botanists and zoologists. The study of nuclear division and the sexual process has led to the location of the hereditary substance in the nucleus, and the establishment of the doctrine of the individuality of the chromosomes, together with their periodic reduction to one-half of the number at some definite point in ontogeny. One of the most important questions pertaining to these theories was the manner in which the reduction is accomplished; and, if we now admit that this problem has found a satisfactory solution, there still remain questions suggested by those steps manifested by the nucleus in the evolution of the chromosomes themselves, as well as other equally important questions that pertain to the manner in which, at least, the more obvious hereditary characters are distributed to the respective sex cells by the individual chromosomes.

When, ten years ago, the writer established the fact that the longitudinal fission of the spirem occurred in the early prophase (i.e. just after synapsis) of the heterotypic mitosis of pollen mother-cells, it was not considered necessary, even by the leading plant cytologists, to make

critical studies of the resting nucleus. The interpretation of the structure and origin of the chromosomes was based upon the form and arrangement of these structures as they appeared just previous to, or upon, the spindle, and their subsequent behaviour during the second or homotype mitosis. The prevailing view of reduction at the time, namely, that this was accomplished during the second division, centred attention largely upon the second and upon the latter part of the first mitosis, with the very important result that the second was not the reducing division. The discovery of the Mendelian principle of the segregation of characters in hybrids stimulated cytologists to a renewed study of the first mitosis with present results.

The more recent study of the presynaptic phase has suggested new problems and new interpretations, not the least important of which is the individuality of the parental chromosomes in somatic cells, and their recognition in the resting nucleus. The writer will now endeavour to show what bearing the facts observed in the plants in question have upon some of the explanations that have been proposed.

The nucleus is almost universally recognized as being in the resting stage when the chromatin is in a finely divided state, consisting of small granules, held in a linin network of delicate threads, in which none of the well-recognized phenomena of mitosis can be detected. Of course, it is not possible, and it may not be necessary, to define the term resting nucleus with strict accuracy. In different plants, and probably under different conditions in the same organ, the degree to which the breaking up into finer granules, or the reticulation of the chromatin has taken place, varies considerably. Sometimes it is certain that just prior to synapsis the reticulation may result in a very finely divided state, in which the granules are perhaps of the same size as, or even smaller than, one-half of the paired chromomeres which appear in the spirem as it merges from synapsis (Figs. 19, 20). In other cases the granules are larger, and may be spoken of rather as lumps or masses than as granules (Figs. 22, 34). This latter condition, especially when the masses or lumps approach in number that of the somatic chromosomes, has been interpreted as indicating the nature in which the individual chromosomes are recognized in the resting nucleus, each lump being a 'prochromosome' or centre of organization of a chromosome. Thus Overton ('05) claims that in the pollen mother-cells of *Thalictrum purpurescens*, *Calycanthus floridus*, *Helleborus foetidus*, and *Campanula grandis*, the number of these masses, or prochromosomes, equals the number of somatic chromosomes. Miyake ('05) suggests a similar interpretation in regard to certain monocotyledonous species, but he does not commit himself to this proposition, stating that it is not easy to determine the number of chromatin lumps, and that in *Lilium* the number of lumps exceeds that of the somatic chromosomes. My own

results seem to show conclusively that there is no definite relation existing between the number of these lumps and the number of somatic chromosomes in *Podophyllum*, *Lilium*, *Tradescantia*, and *Galtonia*, and consequently these plants lend no support to the prochromosome theory.

In regard to the differentiation of the nuclear framework into chromatin and linin, the writer believes that there can be little doubt in this, and he differs from the view expressed by Grégoire and Wygaerts ('03) that all is chromatin. In a later publication Grégoire ('06) has modified his view in this particular, admitting that such a differentiation may exist. It is true that in resting nuclei, in which the strands of the network connecting the chromatin masses are rather thick, these strands retain more of the stain, in which case the difference in appearance between chromatin and linin seems to be small indeed; and, if the iron-haematoxylin stain is used, frequently no difference is at all apparent. On the contrary, in such nuclei as Figs. 17, 19, and 40 of the presynaptic stage, and in Figs. 7 and 25 following synapsis, good staining by both the triple and iron-haematoxylin methods shows beyond doubt that we have two substances sufficiently distinct to justify the designation chromatin and linin. By this statement the writer does not intend to convey the idea that our methods of differential staining, however perfect they may seem, demonstrate completely the difference between chromatin and linin, but to indicate that such difference really exists, leaving the ultimate nature of the two substances to be determined by future research.

This study has also shown that in the same plant the manner of the distribution of the chromatin may be different prior to the synaptic contraction (Figs. 22, 23, 19, and 20). In some cases the lumps remain relatively large, while in others a breaking-up into much finer granules obtains. In all cases, however, the thread on emerging from the balled-up mass is made up of chromomeres in which there is a general uniformity in shape and size. This shows conclusively, that, whatever else may happen within the synaptic mass, this is certain, that there does take place in cases as shown in Figs. 22 and 34 a further reticulation of the chromatin. To this phase of the subject we shall return beyond.

As repeatedly asserted in the foregoing, the chromatin spirem emerges from synapsis, showing for the most part a double row of granules or chromomeres, which I interpret as the result of a longitudinal fission of the chromomeres, and that the entire thread is double seems to be indicated by the divergence in certain instances of the sister threads (Fig. 24). That we do not see all chromomeres paired or of the same size does not argue against the doctrine of a longitudinal fission more forcibly than against the union of two different spirems; and in view of the manner in which the bivalent chromosomes are formed from the spirem, it is reasonable to conclude that, if the longitudinally split thread is not the result of fission, there

is no such fission in either somatic or spore mother-cells, and the only conclusion remaining is that the spirem is formed double under all circumstances. The writer doubts whether any cytologist is prepared at present to defend such a doctrine.

In *Podophyllum*, as the chromatin thread becomes arranged into the hollow and regular spirem, the longitudinal split, as a rule, disappears from sight. Whether the sister threads completely fuse, or become so closely applied only as to conceal the double nature, cannot be stated. The latter alternative is not improbable, especially when we remember that we have to do with two cords composed of a thick fluid substance of exactly similar consistency. The next step is the rearrangement of the spirem which results in the so-called second contraction figure, in which much of the spirem may appear as radiating loops, while the remainder is in the form of an entangled mass near the centre of the nuclear cavity. Cross segmentation now follows, and the rapid shortening of the segments begins. In the formation of the second contraction figure, and from the nature of the chromosomes as they become more or less free, it is clear that each is formed by the approximation of two parts of the spirem that were arranged end-to-end in the thread, and not by two sister segments that first separated laterally and then came together again, or united at one end to form loops, open or closed links, and so on. In *Tradescantia* the case is equally clear, though certain details are more difficult to follow than in *Podophyllum*. The same process obtains in *Lilium Martagon* and *L. candidum*, although in these species, especially in *L. candidum*, a factor enters which tends to confuse greatly the regular course of events, and which has led the writer, together with many others, into error. I refer to the tendency in many cases of the sister threads of the longitudinally split spirem to divaricate rather widely for longer or shorter intervals prior to, or during, the rearrangement of the spirem into the loops. In some cases the divergence of the halves seem to be greater when the loops were more regular, but as to this being a rule cannot be stated with certainty. It should be remembered that a wide divergence of the sister threads does not occur in all cells, and, what is of greatest importance, those which do separate, as described, come together again before cross segmentation, and become so closely applied as to conceal the double nature of the thread. This fact seems to have been entirely overlooked by Berghs ('04). When one looks at Berghs' series of figures (l. c., Figs. 1–8) it seems that he has made out a case that can scarcely be questioned. But he neither describes nor figures the stages showing the second contraction or rearrangement, as illustrated by my Figs. 28, 29, and 30. He has evidently overlooked the fact that the divaricated halves of the spirem come together again before complete cross segmentation. In his (l. c., '04, I) Figs. 2–4 he has shown only isolated portions of the spirem. In order

that his Figs. 3–5 may be convincing, it is necessary to show the relation of such pieces as Figs. 3 *a*, 3 *b*, 3 *c*, and 4 *a*, 4 *b*, and 4 *c* to a large part of the remaining thread in their respective nuclei. Berghs seems to have assumed that the sister threads divaricate, that they do not become closely applied again in the spirem, and that those which form loops and so forth unite at one end after cross segmentation. I am convinced that Overton ('05), Miyake ('05), and Allen ('05) have been led into similar errors. If there should be any question concerning this point in *Lilium*, there can be no doubt in the case of *Podophyllum*, that the bivalent chromosomes are produced by an approximation of two chromosomes arranged end-to-end in the spirem, and even as regards *Lilium* the observed facts seem to admit of no other interpretation.

That the first mitosis in the pollen mother-cells is the reducing division was first asserted by Heuser in 1884 for *Tradescantia virginica*, and ten years later by Korschelt ('95) for an animal, *Ophryotrocha puerilis*, one of the annelids. Speaking of the chromosomes when arranged in the equatorial plate of the first mitosis in the pollen mother-cell, Heuser says: 'Hier sind in der Sternform die Elemente aussergewöhnlich lang,—sie reichen fast von Pol zu Pol. Ihre eigentliche Theilung wird nicht durch Längsspaltung, sondern durch eine nochmalige Quertheilung in der Nähe des Aequators besorgt; dann erst, also nach Anlage der Tochterkerne, erfolgt die Längsspaltung der Tochterstrahlen.'

From this it is evident that Heuser regarded the first, or heterotypic mitosis, as a reducing division, and that the separation of the chromosomes was a cross segmentation and not a separation along the line of longitudinal fission. He was the first also to state that the daughter segments were split lengthwise as they passed to the poles. Heuser gave no figures of *Tradescantia* in the paper cited, and his conclusions were regarded as a mere guess, and consequently little heeded by subsequent observers. The same may be said of Korschelt's account of *Ophryotrocha*, although, judged in the light of what is now known for many animals, he seems to have made out a tolerably clear case. As regards certain worms, however, the results of Foot and Strobell ('05) seem to leave no doubt as to the correctness of this view. Even admitting that Heuser's conclusion was largely in the nature of a guess, and there have been many since 1884, it was, nevertheless, based upon certain correctly observed, though insufficient, data. To show the influence upon investigators of conclusions drawn by eminent specialists from a series of apparently correctly observed and well-organized facts, the writer takes the liberty to recall an incident that occurred when he began the study of mitosis. In 1896, while working in a foreign laboratory, he remarked to a fellow student that, if there was any such thing as a reducing division in higher plants, the same takes place during the first mitosis in the pollen mother-cells. His friend replied

with a look of amusement thus: 'Do you think Flemming has made a mistake in the salamander?' referring to that author's classic paper on the speratogenesis in Salamandra. No, we did not believe that Flemming could have been wrong in the matter in question, and the most eminent cytologists at the time, including the master under whom the writer worked, could not think of a reducing division occurring except in the second or homotypic mitosis. As regards the observers who in recent years have interpreted the first mitosis as a reducing division, holding that the chromosomes separate crosswise and not along the line of the longitudinal split, it may be said without doing any injustice to the ability of these workers, that the evidence set forth by them has not been wholly convincing.

The establishment of the fact that the segments of the heterotype chromosomes (the daughter chromosomes) are split lengthwise during the anaphase, together with a more accurate knowledge of their subsequent behaviour in the reconstruction of the daughter nuclei and during the homotype mitosis, precluded all possibility of a reduction division in this mitosis in so far as the higher seed plants are concerned. Furthermore, the Mendelian principle of segregation of characters in certain hybrids seemed to demand a reducing division, and whether this principle be correct or not, it has certainly accomplished much good in stimulating a search for a reducing and a qualitative mitosis. Botanists, therefore, directed their renewed attention to the first mitosis with present results. Although Farmer and Moore were probably the first to present the most convincing series of facts in behalf of the reduction in number taking place in the first or heterotypic mitosis, yet the credit for this explanation belongs by right of priority to Heuser for the higher plants and probably to Korschelt for animals.

The belief in the individuality of the chromosomes is now almost, if not quite, universal among cytologists and students of heredity. A consideration of this doctrine in the light of the rapidly increasing data, and the very general agreement among the various observers concerning the manner in which the reduction in number of the chromosomes is accomplished, brings the investigator face to face with very important and far-reaching problems. Among these the question of the recognition of the identity of the chromosomes in the resting nucleus has received attention by recent workers, and the result has been the suggestion of the prochromosome idea. In certain plants there is a tendency of the chromatin to form lumps or masses in the resting condition, in which there is often a general uniformity in size, and when the number of such lumps approaches the number of somatic chromosomes, each mass has been looked upon by some as representing a prochromosome, or the centre of organization of a chromosome (Rosenberg, '04; Overton, '05). In the light of the facts presented in this paper, it is evident that the plants here considered lend no support to such a view. The chromatin does not always appear in the

form of definite and well-defined masses in the same plant, and when such occur their number far exceeds the number of chromosomes. When all the facts are duly considered, we must admit that there is nothing in the resting nucleus that we can look upon as representing a chromosome, for all identity of such bodies is lost. In the opinion of the writer, it is better not to associate the idea of identity with the chromosomes as these are recognized upon the mitotic figure, but this idea should be connected with smaller units. Whether these units be regarded as hypothetical or as objects capable of observation may make little difference in the construction of a theory, but in order to eliminate as much as possible the purely problematic factors, it seems to me best to select as our unit, to which we ascribe individuality, some part of the chromatin capable of objective demonstration in the resting nucleus. In so doing I have selected the term pangen of the de Vriesian terminology chiefly because this term has been used in a similar sense by others (Strasburger, '05). Differing somewhat from the meaning put into the term by Strasburger, I shall apply the term pangen to the small but distinguishable particles that compose the chromomeres. In the spirem, as it emerges from the synaptic mass (Fig. 25 *a*), the chromomeres are seen to be made up of smaller granules. These smaller granules, which I shall speak of as pangens, can be recognized as well in the resting nucleus. It may be said, of course, that each of these granules composing a chromomere can be regarded as made of still smaller bodies, and why not apply the term pangen to these smaller bodies as others have done? I have not done so because, if we apply the term to objects too small for objective demonstration, it would be just as well to apply it to molecule or atom. Furthermore, in using the word pangen to designate the small but recognizable granules composing the chromomeres, cytologists will have some definite idea of what is meant. We shall understand, therefore, that each chromomere is composed of a number of pangens, leaving the number indefinite, inasmuch as all chromomeres are not the same size, and do not appear to be made up of the same number of these granules or particles; and it may not be possible in any case to determine the number of pangens in a given chromomere. Each chromosome will then be composed of a number of chromomeres, also variable in number. The use of a long series of terms, such as pangen, pangenosome, gamosome, and zygosome, as proposed by Strasburger, seems to me to confuse rather than to elucidate, and for the sake of simplicity it is better to retain the terminology already established for the larger collections of units. Each pangen may be conceived as bearing one or more qualities, but how many and what qualities are purely matters of theory.

Turning now to the idea of hereditary qualities or characteristics, the following questions present themselves. Do the chromosomes of each

gamete possess all the characters of the species? Or does the sperm, for example, contain characters not possessed by the egg? Or does each gamete contain all the characters of the species, with some character, or characters, having a stronger potentiality in one gamete than in the other? Among plants should any greater significance be attributed to variability in the size of the chromosomes in any individual than to the variability in the number of chromosomes in different species? A perusal of the recent literature on the subject shows one that the majority of theoretical discussions concerning the transmission of hereditary characters embodies some answer to the above questions, which is either assumed in the beginning or developed in the course of the argument.

Probably the prevailing view among botanists is that, in the chromatin of each parent or in each gamete, all the qualities are represented, for upon no other assumption does it seem possible to explain the parthenogenetic development of an egg into an individual similar to one arising from fecundation. Furthermore, in the case of an homosporous fern, for example, the spores contain only one-half the number of chromosomes of the sporophyte that bears them, yet the prothallia resulting from these spores produce both male and female gametes, which upon union develop into a sporophyte similar to the parent sporophyte. From these and numerous other cases, the only conclusion that can be drawn is that the reduced number of chromosomes, that is, those of any one gamete, contain all the characters of the individual. Strasburger ('05, p. 59) seems to believe that the reduction division itself signifies the conclusion that before reduction all the primordia are present in duplicate, that is, double, while after reduction all occur single. 'Dass diese reduzierte Zahl die Anlage für alle Merkmale des gegebenen Organismus in sich fasst, geht aus dem Wesen der Reductionsteilung hervor. Vor der Reductionsteilung waren alle Anlagen doppelt vertréten, nach der Reductionsteilung sind sie einfach vorhanden.'

In order that we may comprehend the significance of this doctrine, and to be able to draw our own conclusion, it is necessary to examine the data during every step in the reducing division. In the resting stage of the microspore mother-cell of *Podophyllum* or *Lilium*, for example, we can recognize definitely, let us say, only pangens, and while we cannot follow in every detail the construction of the spirem, and hence the chromosomes, out of these pangens, we may draw certain definite conclusions from those details that admit of objective demonstration, while other interpretations must remain problematical. Prior to the contraction into the balled-up mass, the chromatin may be rather finely and evenly distributed in the nuclear framework (Fig. 19). These fine granules may be regarded as pangens. On the other hand, the pangens may be collected into larger masses, each mass being usually much larger than a chromomere (Figs. 16,

17, 18). The contracted mass loosens up as the regular spirem, in which may be recognized the series of chromomeres held in the linin matrix. These chromomeres are for the most part paired, which I think is due to the longitudinal splitting of single chromomeres. The paired chromomeres vary somewhat in size, but on the whole there is much uniformity. Some of the chromomeres are, however, not paired, at least so far as can be made out, and many of the unpaired ones are smaller than the average. Now the question is, what happens in synapsis? According to Strasburger ('05, p. 37), there takes place in the synaptic mass a rearrangement by which homologous pangens are brought together. 'Meine Ansicht ist jetzt also die, dass durch die Wechselwirkung der beiden Gamosomen ihre Pangene eine bestimmte Orientierung erfahren, so dass sie bei der darauf folgenden Streckung dieser Gamosomen eine übereinstimmende Aufeinanderfolge erhalten. Dieser vorbereitende Schritt hätte somit das Ergebniss, die homologen Pangene an der gestreckten Gamosomen einander unmittelbar zu nähern.' The bringing together of homologous pangens means that every maternal pangen is brought near to its corresponding paternal pangen. Since Strasburger regards the longitudinal fission of the spirem as being due to a union of two parental threads rather than a fission, one naturally infers that he understands that the homologous groups of pangens are brought together side by side.

We cannot, of course, determine what takes place in synapsis, but it may be possible that some kind of a rearrangement occurs. In those cases in which the larger masses of chromatin are present just prior to the contraction, it is certain that these larger masses are broken up into much smaller ones before the loosening up process begins. If any rearrangement takes place, this must happen either during the balled-up condition or earlier; for it is less probable that a rearrangement and an exchange of pangens should be brought about in the longitudinally split spirem. In view of the orientation of the parental chromosomes in the spirem, that is, end-to-end, as set forth in this paper, it seems to me that, if some sort of rearrangement or interchange takes place in synapsis, this must be different from that held by those who regard the parental chromosomes placed side by side in the spirem. To me it seems that in synapsis or earlier the pangens of like affinity, or those bearing like characters, are brought together into chromomeres. These chromomeres are then organized into chromosomes. Each chromosome is accordingly composed chiefly of homologous chromomeres, which become arranged end-to-end, and which split lengthwise to form the paired chromomeres. A longitudinal fission means an equal distribution of homologous chromosomes, and this we know is accomplished during the second or homotypic mitosis in all plants thus far thoroughly investigated.

If it is assumed that all chromosomes are not alike in every quality,

then all pangens are not alike. There is no definite evidence, as yet, among plants that any particular chromosome is entirely different from others, but there is at the same time every reason to believe that each gamete may contain all, or nearly all, of the parental qualities. It is also probable that these qualities are present in different degrees of potentiality in the different chromosomes. The theory of sex determination developed in the insects (Wilson, '06, and others) cannot be applied with equal force to plants, for no accessory chromosome, or probable sex determinant, has been observed in the plant kingdom. We may, however, reasonably attribute to the chromosomes in plants certain different qualities falling within narrow limits, and such an assumption may be based upon observed facts. It is seen that, in the spirem in which the individual chromomeres are demonstrable (Figs. 25, 25*a*), all of these are not paired, especially in the case of the smaller ones. The probabilities are that these smaller granules may become a part of one chromosome or another according to the rules of chance, or according as certain qualities of a group have a stronger affinity for each other than for others. If we are in any way justified in ascribing some individual quality or qualities to these apparently unpaired granules, then we are justified in concluding that some chromosome or chromosomes are to that extent hereditarily different from others. Otherwise, what value can a reducing division have further than that of a mass reduction? Of course, we may say also with a large degree of probability that the nuclei of the gametophyte differ from those of the sporophyte only in the amount of hereditary substance, and not a difference in the hereditary qualities of that substance.

In regard to placing any great significance in the fact that in some plants certain chromosomes are smaller and some larger than others, it seems to me that the constancy of such difference is not sufficient to warrant any definite conclusion at present, especially when the source of error in estimating the size of the chromosomes is considered, and also in the additional fact that, in many plants, one of the most conspicuous things is the difference in size of the chromosomes in similar cells. If we transfer the idea of individuality from the chromosome to the pangen, the significance of difference in size seems to be greatly diminished. In this connexion the question of the significance of the number of chromosomes in any plant arises. Why should one plant reveal only sixteen chromosomes, another twenty-four, and still others larger numbers? In *Podophyllum* and *Lilium*, which possess relatively large cells and a large amount of chromatin, we have relatively small numbers but large chromosomes. In other plants, as perhaps in the majority of Dicotyledons, there are usually smaller cells, a smaller quantity of chromatin, and a relatively large number of small chromosomes. Just what relation may exist between the size of the cell, the mass of chromatin, and the size and number of chromo-

somes in plant cells has not been ascertained, and it is apparent at the outset that accurate results will be difficult to obtain. That which is of greatest theoretical importance is the fact that the number of chromosomes in any species is constant. The number, however, whether large or small, may be due to purely mechanical causes, but whatever the determining factors may be, it is certain that these are fixed by heredity, and the difference in the size of the chromosomes in any plant may have no greater importance than the difference in the number of chromosomes in different plants. The difference in size of the chromosomes in any plant, whenever it is found to occur with a high degree of regularity and constancy, is, of course, very suggestive, and the writer does not wish to be construed as under-estimating the value of such facts, or the usefulness of theories based thereon.

In connexion with any theory of the individuality of the pangens and their distribution in reduction, there is another very important fact or series of facts to be taken into consideration. In the entire plant kingdom, with the one known exception of the genus *Pinus*, the gamete nuclei fuse in fecundation while in the resting condition, or when the chromatin in each nucleus is in the more finely divided state. It is not possible to recognize in the fusion nucleus male and female chromatin, nor is any such recognition possible in the nuclei of the sporophyte generation. This being the case, what ground is there for assuming that the male and female chromatin remain distinct throughout this generation, being merely associated in the cells? If there be any rearrangement, exchange, or pairing of homologous pangens, why should not all this take place when the nuclei fuse, rather than at the close of the cycle when the reduction in number of the chromosomes occurs? In the division of the sporophytic nuclei there is in many cases, at least, a spirem formed, and this spirem separates into double the number of chromosomes, one-half having been furnished by the egg and the other half by the sperm. In this spirem we must admit that the chromosomes are arranged end-to-end, if we believe in the individuality of the chromosomes, for each division is equational. Thus arranged, are all paternal chromosomes united end-to-end to form one continuous half of the spirem, while the other half is formed by the maternal chromosomes? Or do the parental chromosomes alternate with each other in the spirem, or merely become arranged according to the rules of chance? If the parental chromosomes are arranged end-to-end in somatic cells of the sporophyte, why should they be not so arranged in the heterotypic spirem rather than side by side, as those who maintain that the double spirem is formed by the union side by side of two spirems. In this connexion it is important to remember that the univalent chromosomes which show the longitudinal split during the anaphase of the heterotypic mitosis do not form a double spirem

in those cases in which a continuous spirem is present in the daughter nuclei (*Podophyllum, Lilium*), but the sister segments unite end to end in this spirem.

The doctrine of the individuality of the chromosomes requires the assumption that the reduced number contains all the qualities of the species, else how is it possible to explain the fact that the monoecious prothallium of a homosporous fern, which arises from a spore containing only one-half the sporophytic chromosomes, produces by the union of gametes a sporophyte identical with the parent sporophyte? The same applies to cases of true parthenogenesis. The fact that the eggs of such a fern do not develop into sporophytes without the union with sperms would seem to be due merely to the lack of quantity of chromatin rather than to the absence of any quality. The assumption that the chromatin of each spore contains all the necessary characters of the species seems quite essential. Yet, on the other hand, the reducing division cannot be qualitative unless some of the chromosomes contain pangens that are either not possessed by others at all, or, if possessed by all, then certain pangens must contain qualities in a greater degree of potentiality. Since any two individuals of the same species have many more qualities in common than those that are different, it follows that the chromatin would consist chiefly of pangens representing these common qualities, while the comparatively few characters of difference would be represented by a relatively small number of pangens. Moreover, the pangens representing these relatively few characters would not only be different from the rest, but their affinity for any of the numerous pangens might diminish as the qualitative difference became greater. It is also conceivable that the pangens which represent certain special or widely different qualities may have a stronger affinity for some of the more numerous pangens than for others, and in this way there could be brought about a localization of the special pangens. Consequently, in the phylogenetic development of the race a more permanent association might result between certain groups of pangens and those bearing the special qualities. In some such manner certain chromosomes might have become qualitatively different from others. The pangens representing these most markedly different qualities may be conceived as coming together in one chromosome, or as distributed among different chromosomes. The foregoing may be made clearer by an illustration. The greatest structural and functional differences between any two sexually differentiated individuals, or members of an individual, of the same species is probably that of sex; and it is possible that the pangens representing sex are those that differ most from all others. This difference may be conceived as being so great that such pangens may unite to form a distinct chromosome, or sex-differential, as seems possible in the case of certain insects. However, the pangens representing these special characteristics, whether they pertain

to sex or some other distinct quality, may be present in one, or distributed among several chromosomes. It does not necessarily follow that any given chromosome should always contain these pangens; that is to say, there is among plants no ground for the conclusion that any one chromosome contains certain qualities, and only those, throughout the history of the species.

It is worthy of note here that in *Marchantia polymorpha*, whose spores produce both male and female gametophytes, these thalli retain their unisexual character when propagated vegetatively by gemmae. Noll[1] is reported to have cultivated both thalli by means of gemmae for over thirty generations, and under varying conditions of growth, without being able to change the sexual character of either strain. In a homosporous fern, which the writer now has under investigation, the spores produce small prothallia that are strictly male, and large ones that bear archegonia. The gametophyte of this fern is reported in recent literature to be strictly dioecious, the statement being made that the large prothallia never bear antheridia. Out of a number of the larger prothallia bearing archegonia, selected at random from a pure culture, about 85 per cent. were pure females and 15 per cent. hermaphrodite. Investigation is in progress to determine the percentage of spores that produce strictly male prothallia, and also the ratio of pure females to hermaphrodite forms among the large so-called female gametophytes. At present my study has not proceeded far enough to justify any statement beyond that made in the foregoing. The commonly accepted view is that by sowing the spores thickly, male prothallia are produced in greater abundance or almost exclusively, while in the less crowded cultures affording better nutrition a larger number of spores will develop into female gametophytes. Efforts will be made in the experiments to determine more accurately, if possible, whether the sex of the prothallia is influenced or determined by conditions of growth.

If the studies under consideration bear out the conclusion obtained from an examination of a very limited quantity of material, it would seem that in the fern in question some spores are strictly male, some purely female, while others contain male and female characters with the female greatly predominating. This condition can be explained in the light of the theory advanced in this paper by assuming that some spores possess a predominance of strictly male pangens, others female, and still others pangens of both sexes with the female predominating. Neither in the spore-bearing cells of *Marchantia* nor in those of the fern in question has anything comparable to an accessory chromosome, or special sex-differential, been found, and, as in the case of the higher plants mentioned in the preceding pages, it seems not impossible that the smaller granules observed in the spirem, which are not included in any of the chromomeres of a larger and more uniform size, may represent almost exclusively male

[1] Reported by Blakeslee ('06).

or female pangens or those bearing other distinct qualities. It seems reasonable also that the sexual differentiation observed in certain moulds (Blakeslee, '06) may be explained in a similar manner.

In conclusion, I think it is not mere speculation to assert that in plants of a low degree of differentiation, the hereditary determinants or pangens are correspondingly much less specialized; but as differentiation along the lines of both vegetative and reproductive structures and functions became more and more pronounced in phylogenetic development, it is only reasonable that the pangens should undergo a corresponding differentiation. And in the higher organisms where certain sharply defined and fundamental characteristics appear, as, for example, those of sex, it follows that these important and fixed characteristics should be represented in the hereditary substance by pangens that are as distinct and as sharply defined from other pangens as the characters in question are different from all other characters of the individual. The question then to determine is whether such pangens are equally distributed among all the chromosomes, or whether any of the chromosomes are qualitatively different. To arrive at any trustworthy conclusions, it is necessary that experimental studies carried out with this end in view must go along with a cytological study of the chromatin.

Summary.

The resting nucleus of the pollen mother-cell consists of a linin net in which the chromatin is held, either in the form of very fine granules of uniform size, or in larger aggregations or lumps which are composed of smaller granules. The chromatin granules or lumps are often evenly distributed within the nucleus; frequently there is a tendency toward a peripheral arrangement of the chromatin, leaving the centre freer from the granules or lumps. One or more nucleoli are present. Prior to synapsis there may be formed, in some cases in *Lilium Martagon*, a very delicate, spirem-like threadwork in which the chromatin occurs as a single row of small chromomeres; in other cases the chromatin did not seem to become so finely divided, the nuclear framework, with larger chromatin lumps, passing directly into synapsis.

Synapsis consists in the contraction of the entire nuclear threadwork into a compact and dense ball, which usually lies close to the nuclear membrane. The position of the contracted mass in the nuclear cavity does not seem to be determined by gravity. The nucleolus may or may not be included within the contracted mass. The contraction of synapsis represents the longest pause in the mitotic process. There is no union of two spirems in synapsis.

The synaptic mass on loosening up forms the hollow spirem, which is double, being due to a longitudinal splitting and not to the lateral union of two spirems. In *Podophyllum* the loose spirem is of a uniform thickness

and rather regularly arranged. In it all traces of the longitudinal fission is concealed, save in exceptional cases.

In *Lilium* the arrangement of the spirem is less regular and less easily followed. The longitudinal fission is as a rule also obscured.

The hollow or loose spirem is followed by a rearrangement of the thread which has been called the 'second contraction'. This second contraction consists in the arrangement of a large part of the spirem into loops that tend to radiate from a closely entangled central mass of the thread. The loops are formed by the proximation of the parallel portions of longer turns of the spirem. In *Lilium*, as the rearrangement into loops takes place, it frequently happens that the halves of the longitudinally split spirem tend to divaricate rather widely in places. Later, these divaricated portions come together again, and the fission is obscured. During the second contraction the cross segmentation begins. The free ends of the loops may lie near the centre with the head or bend towards the nuclear membrane, or the reverse may be true. The parallel sides of the loops are frequently closely applied and twisted about each other. Each loop represents a bivalent chromosome, each parallel part being a single chromosome. The two parallel parts, or chromosomes, were arranged tandem, or end-to-end, in the spirem. All of the bivalent chromosomes do not arise as loops; the two parts or segments may approximate in various positions. This fact accounts for the variously shaped chromosomes observed in later stages. The more entangled parts of the spirem form the curiously curved and twisted bivalent chromosomes. The rearrangement or second contraction is a regularly occurring step in the heterotype mitosis in all the plants examined.

As the bivalent chromosomes separate from the second entanglement, they tend to become distributed within the nucleus and frequently near the periphery. In *Tradescantia* and *Galtonia*, there is in many cells a marked tendency for some of the segments to adhere end-to-end, forming chain-like rows of sausage or kidney-shaped pieces. Whether this is due to incomplete cross segmentation, or to a re-adherence subsequent to cross segmentation, cannot be stated. Other chromosomes in the same nucleus may have the two parts so arranged as to form open or closed links, or these may lie side by side as two short thick pieces. In all the plants studied, the two members of each bivalent chromosome were not side by side in the spirem, representing the halves of the longitudinally split thread, but they were two different parts of the spirem that were arranged end-to-end in the chromatin thread.

As the two members of the bivalent chromosome separate in metakinesis or during the anaphase, each is seen to be split longitudinally, and I am convinced that this is the original longitudinal split seen in the early prophase. This longitudinal split represents the line along which the

daughter segments will separate in the second mitosis. The first or heterotypic mitosis is a reducing division; the second or homotype division is equational. To what extent the heterotype division is qualitative is an open question.

Literature Cited.

Allen, C. E. ('05): Nuclear division in the pollen mother-cells of *Lilium canadense*. Ann. Bot., xix, 189–258, 1905.

———— ('05): II. Das Verhalten der Kernsubstanzen während der Synapsis in den Pollenmutterzellen von *Lilium canadense*. Jahrb. f. wiss. Bot., xlii, 72–82, 1905.

Atkinson, G. F. ('99): Studies on reduction in plants. Bot. Gaz., xxviii, 1–26, 1899.

Berghs, J. ('04): Formation des chromosomes hétérotypiques dans les sporogénèses végétales. I. Depuis le spirème jusqu'aux chromosomes mûrs; La Cellule, xxi, 173–88, 1904. II. Depuis la sporogonie jusqu'au spirème définitif; l. c., xxi, 383–94, 1094. III. La microsporogénèse de *Convallaria maialis*; l. c., xxii, 43–9, 1904.

———— ('05): La microsporogénèse de *Drosera rotundifolia*, *Narthecium ossifragum*, et *Helleborus foetidus*; l. c., xxii, 141–60, 1905.

Blakeslee, A. F. ('06): Differentiation of sex in thallus, gametophyte and sporophyte. Bot. Gaz., xlii, 161–78, 1906.

Farmer, J. B., and Moore, J. E. S. ('05): On the Maiotic Phase (reduction division) in animals and plants. Quart. Journ. Mic. Sci., xlviii, 489–556, 1905.

Farmer, J. B., and Shove, Dorothy ('05): On the structure and development of the somatic and heterotype chromosomes in *Tradescantia virginica*. Quart. Journ. Mic. Sci., xlviii, 559–69, 1905.

Foot, K., and Strobell, E. C. ('05): Prophase and metaphase of the first maturation spindle of *Allolobophora foetida*. Am. Journ. Anat., iv, 199–243, 1905.

Grégoire, V., and Wygaerts, A. ('03): La reconstitution du noyau et la formation des chromosomes dans les cinèses somatiques. La Cellule, xxi, 7–74, 1903.

Grégoire, V. ('05): Les cinèses de maturation dans les deux Règnes. Revue critique de la littérature. La Cellule, xxii, 221–376, 1905.

———— ('06): La structure de l'élément chromosomique au repos et en division dans les cellules végétales. La Cellule, xxiii, 311–53, 1906.

Heuser, F. ('84): Beobachtungen über Zellkerntheilung. Bot. Centralbl., xvii, 1884.

Korschelt, E. ('95): Ueber Kerntheilung, Eireifung und Befruchtung bei *Ophryotrocha puerilis*. Zeit. f. wiss. Zool., lx, 543–688, 1895.

Miyake, K. ('05): Ueber Reductionsteilung in den Pollenmutterzellen einiger Monokotylen. Jahrb. f. wiss. Bot., xlii, 83–120, 1905.

Mottier, D. M. ('97): Beiträge zur Kenntniss der Kernteilung in den Pollenmutterzellen einiger Dikotylen und Monokotylen. Jahrb. f. wiss. Bot., xxx, 169–204, 1897.

———— ('98): Ueber das Verhalten der Kerne bei der Entwickelung des Embryosacks und die Vorgänge bei der Befruchtung. Jahrb. f. wiss. Bot., xxxi, 125–57, 1898.

———— ('99): The effect of centrifugal force upon the cell. Ann. Bot., xiii, 325–61, 1899.

———— ('03): The behaviour of the chromosomes in the spore mother-cells of higher plants and embryosac mother-cells. Bot. Gaz., xxxv, 250–82, 1903.

———— ('05): The development of the heterotype chromosomes in pollen mother-cells. Bot. Gaz., xl, 171–7, 1905.

Overton, J. B. ('05): Ueber Reduktionsteilung in den Pollenmutterzellen einiger Dikotylen. Jahrb. f. wiss. Bot., xlii, 121–53, 1905.

Rosenberg, O. ('04): Ueber die Individualität der Chromosomen im Pflanzenreich. Flora, xciii, 251–9, 1904.

SCHAFFNER, J. H. ('97): The division of the macrospore nucleus. Bot. Gaz., xxiii, 430–52, 1897.
———— ('06): Chromosome reduction in the microsporocytes of *Lilium tigrinum*. Bot. Gaz., xli, 184–90, 1906.
STRASBURGER, E. ('04): Ueber Reductionsteilung. Sitzber. König. Preuss. Akad. Wiss., xviii, 587–614, 1904.
———— ('05): Typische und allotypische Kerntheilung. Jahrb. f. wiss. Bot., xlii, 1–71, 1905.
WILSON, E. B. ('06): Studies on chromosomes. III. The sexual differences of the chromosome-groups in Hemiptera, with some considerations on the determination and inheritance of sex. Journ. Exp. Zool. iii, 1–40, 1906. Important zoological literature is here cited.

EXPLANATION OF PLATES XXVII AND XXVIII.

Illustrating Professor Mottier's paper on *Heterotypic Chromosomes*.

All figures were drawn from sections with the aid of the Abbé camera lucida and with Zeiss apochromatic immersion 2 mm., apert. 1.40, and compensating ocular 12. Magnification × 1800.

Podophyllum peltatum.

Fig. 1. Pollen mother-cell prior to synapsis. The chromatin is distributed in the linin net in the form of granules of medium size; the threadwork of the cytoplasm is very distinct.

Fig. 2. A nucleus of pollen mother-cell from the same anther. The chromatin appears in larger masses or lumps, having chiefly a peripheral position; the nucleolus lies in the centre of the cavity which is freer from the linin net.

Figs. 3 and 4. Cells just prior to synapsis, taken from the same anther loculus. In Fig. 3 the chromatin granules are collected into shreds, suggesting a spirem-like condition; in Fig. 4 the chromatin consists of small granules evenly distributed throughout the linin net. The cytoplasm is finer mesh than in Fig. 3.

Fig. 5. Complete synapsis. The entire nuclear contents are contracted into a dense mass. The cells show a complete connexion at this stage.

Fig. 6. The synaptic mass beginning to loosen up. The longitudinal fission is seen in the free parts of the spirem. At this stage the cells show the first signs of rounding off at the corners.

Fig. 7. The loose or hollow spirem. At certain places the halves of the longitudinally split spirem diverge slightly.

Fig. 8. A later stage of the hollow spirem; the section includes a large part of the nucleus. The spirem consists of a rather smooth cord of uniform thickness, in which, as a rule, no trace of the longitudinal split can be seen. In this figure the spirem has begun to arrange itself into the long loops. Between Figs. 7 and 8 we have a more regular spirem, showing fewer loops.

Fig. 9. The rearrangement or second contraction. The two limbs of each loop have approximated side by side, and some have become greatly twisted about each other. In some cases the longitudinal split can be seen in each limb of the loop. Cross segmentation has begun.

Fig. 10. Second contraction complete, as is also the cross segmentation. The bivalent chromosomes tend to radiate from the centre where their inner ends are much entangled. Either the two free ends or the head of the loops may lie toward the nuclear membrane. Preparation for the spindle formation is indicated by the presence of kinoplasmic fibres radiating from the nucleus.

Fig. 11. A later stage in formation of spindle, showing several bivalent chromosomes scattered within the spindle complex. The longitudinal split in each segment is no longer apparent.

Fig. 12. A later stage; chromosomes being arranged in nuclear plate.

Fig. 13. Mature spindle, showing forms of chromosomes commonly observed.

Fig. 14, *a*, *b*, *c*, *d*. Some of the usual forms of chromosomes met with.

Lilium Martagon.

Fig. 15. Nucleus of a young pollen mother-cell at the close of the last sporogenous division. The chromatin lumps composed of smaller but distinct granules show a tendency to be grouped about the several nucleoli, from which thicker and thinner strands of linin tend to radiate.

Fig. 16. A later stage in presynaptic growth. The chromatin has increased in quantity, and the nucleus has become greatly enlarged.

Fig. 17. Similar to the preceding; chromatin uniformly distributed in lumps or masses.

Fig. 18. A similar stage. The nucleus became differentiated in such a way as to show both the chromatin and the linin of the lumps.

Fig. 19. Beginning of synapsis. Here the chromatin, in the form of finely divided granules of rather uniform size, is arranged in a spirem-like net, which seems to show some orientation with reference to one of the flattened nucleoli. The nucleolus or nucleoli in such nuclei are flattened against the nuclear membrane (the sickle stage of older literature).

Fig. 20. A similar condition of the chromatin, showing a further step in the contraction.

Fig. 21. Complete synapsis of such nuclei.

Fig. 22. Nucleus undergoing synapsis in which the chromatin remained in the larger lumps.

Fig. 23. Complete synapsis of this condition.

Fig. 24. The synaptic mass loosening up. The double row of chromomeres show that the thread is longitudinally split or undergoing this process.

Fig. 25. The hollow spirem just after synapsis, showing longitudinal fission. All chromomeres do not seem to be paired. Free ends are due to cutting.

Fig. 25 *a*. A portion of the thread more enlarged.

Lilium candidum.

Fig. 26. Similar, or a little later than Fig. 25. The thread stained more uniformly, and the chromomeres are not readily seen. This figure includes more of the nucleus than Fig. 25.

Fig. 27. Hollow spirem in which the rearrangement into loops and the central entanglement have begun. At this stage the longitudinal halves of the spirem tend to divaricate for longer or shorter distances. This is not true in all nuclei.

Fig. 28. A later stage. The spirem is still quite slender, having shortened and thickened only a very little. The formation of the loops is evident, and the central entanglement about the nucleolus is more pronounced.

Figs. 29, 30, 31. Later stages. The parallel sides of loops have come closer together. The longitudinal halves of the thread that may have divaricated in an earlier stage have come together again, so that traces of this fission are becoming more difficult to observe. Cross segmentation now takes place. In Fig. 30 this may be complete; in Figs. 29 and 31 the central entanglement is too complicated to admit of a clear view. At this stage the spirem is usually as entangled as Fig. 29 and even more so. From the stage of Fig. 30, i. e. after cross segmentation, the greatest shortening and thickening of the chromatin takes place.

Fig. 32. The bivalent chromosomes are beginning to separate and to distribute themselves within the nucleus.

Fig. 33. Chromosomes distributed. The formation of the spindle begins at this stage. The loop-like character of the majority of the chromosomes is still seen. In this and earlier stages delicate linin threads extend out from the chromosomes that connect them with each other and with the nuclear membrane.

Tradescantia virginica.

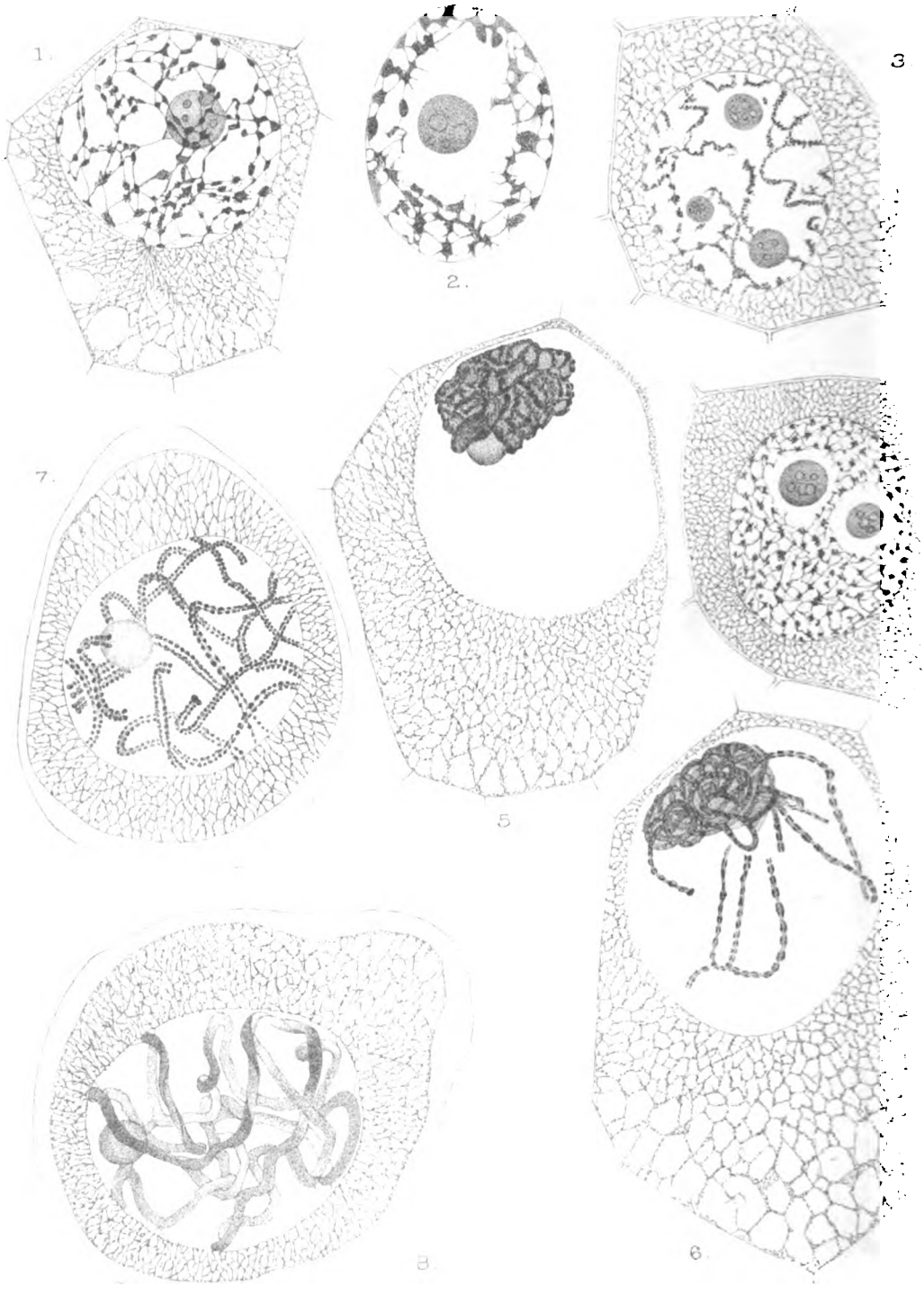
Fig. 34. Presynaptic stage; chromatin in larger or smaller groups or lumps.

Fig. 35. The loosening up of the synaptic mass. The loosening spirem presents a rather regular arrangement, the so-called 'bouquet' stage. The longitudinal fission is seen in places.

Figs. 36 and 37. The second contraction figure. Cross segmentation almost complete in 37. All traces of the longitudinal splitting have disappeared from sight.

Figs. 38, 39. The chromosomes distributed in nucleus prior to formation of spindle. The segments or univalent chromosomes are frequently attached end-to-end, forming a zig-zag row of sausage-like pieces.

Fig. 40. A stage in the formation of multipolar spindle. The chromosomes are crowded together somewhat, a usual phenomenon at this stage. Some still show the end-to-end attachment. This persists in some cases even to the metakinesis (see author's figures, '03).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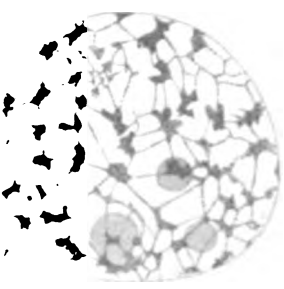

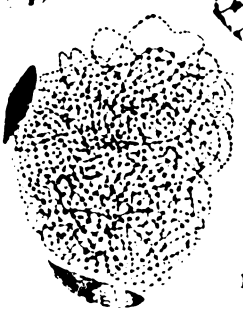

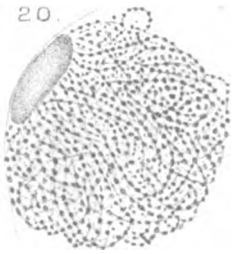

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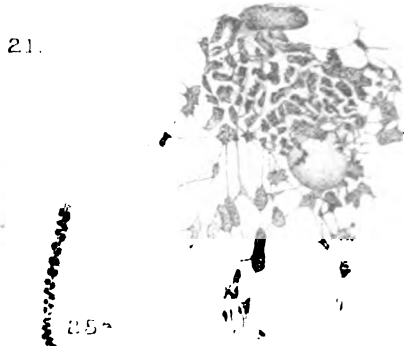

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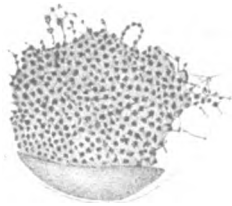

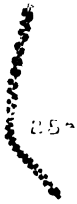

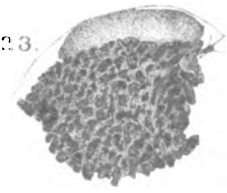

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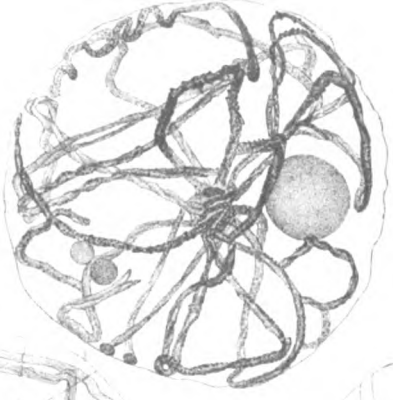

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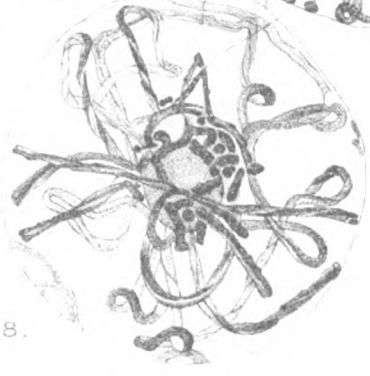

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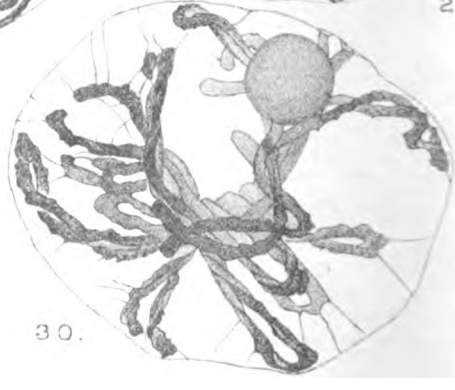

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On the Sexuality and Development of the Ascocarp in Lachnea stercorea, Pers.

BY

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With Plates XXIX and XXX.

THE recent discovery in *Humaria granulata* (Blackman and Fraser (4)) of a reduced type of fertilization, brought about by the fusion in pairs of the nuclei of the female coenogamete, rendered advisable the investigation of other related forms with a view to ascertaining whether such a process be of common occurrence.

Various Discomycetes, growing on wood and on dung, were therefore examined, and a well-developed ascogonium was found in *Lachnea stercorea*.

Lachnea stercorea, Pers. (=*Peziza stercorea*, Pers.), is a small orange Discomycete, occurring during the winter and spring on cow-dung. It is about 4 mm. in diameter, and is furnished with numerous long hairs. The material used was always allowed to develop upon its natural substratum, as in view of the conflicting results obtained by Harper (17) and Dangeard (8) on *Pyronema confluens*, it seemed wiser to avoid any unnatural conditions in dealing with a case of doubtful or reduced sexuality.

Methods. Material was fixed in chromacetic acid and in various strengths of Flemming's fluid, Flemming's weaker fluid, diluted with an equal volume of water, being found the most satisfactory. Sections were usually cut 5μ in thickness, and were stained either with Flemming's triple stain or with Heidenhain's iron haematoxylin, followed by eosin. This stain proved very successful. The slide after treatment with haematoxylin was covered with a fairly strong solution of eosin in clove oil, which was washed off after a few seconds with clove oil, followed by cedar oil. The cell walls of the ascocarp were by these means stained bright red, and the definition of the nuclei was also enhanced. Preparations mounted in Canada Balsam sometimes faded rapidly, but they kept well in Damar Lac or in Gumthus.

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Germination of the Spores.

The spores were placed in various fluids at a temperature of about 15° C., but without result. In nature the spores probably pass through the body of the cow, and an attempt was therefore made to imitate normal digestion; spores were placed successively in saliva, in gastric juice (a few drops of liquor pepticus of Benger in 0·2 per cent. aqueous solution of hydrochloric acid), in pancreatic juice (one part of Benger's liquor pancreaticus to two parts of 1 per cent. aqueous solution of sodium carbonate), and in watery extract of cow-dung. The spores were left in each of the first three fluids for 3–4 hours, and in the dung extract for considerably longer, at a temperature of 38° C. They were then transferred in dung extract to slides, where they were allowed to cool gradually. The spores, which, before treatment, appeared thick-walled and hyaline (Fig. 1 *a*), showed an increase in size, thin walls, granular contents, and one or two large vacuoles (Fig. 1 *b*). Later the vacuoles became more numerous, and the spores still larger, and, about two days after the beginning of the experiment, germinations were observed, a germ-tube passing out from each end of the spore.

In order to ascertain which of the fluids used was effective in germination, they were now employed separately, and spores were placed in dung extract at a temperature of 38° C. for several hours and then mounted as before. Germinations took place, the mycelium being, in certain cases, well developed and showing branches (Fig. 1 *c*). Spores placed in distilled water, but otherwise treated exactly like those in dung extract, did not show even the changes in size and contents preliminary to germination.

The alkalinity of a sample of dung extract was now ascertained, and it was found to be approximately that of a centinormal solution. Spores were placed, therefore, in decinormal and in centinormal solutions of sodium carbonate, and in a solution half the strength of the latter $\left(\frac{N}{200}\right)$. They were kept for about 30 hours at 38° C., and then mounted and allowed to cool. The spores in the $\frac{N}{10}$ solution were somewhat shrunken and distorted, no doubt by plasmolysis, and showed no further changes. In the other two solutions they were thin walled and vacuolate. After various attempts had been made, germinations were obtained in a $\frac{N}{100}$ solution, but they were rare, owing, probably, to lack of food-material, and, in more than one case, the germ-tubes from a spore germinating in the ascus were found running in the epiplasm, and no doubt obtaining nourishment from it. (Fig. 2.)

The results of these and other experiments are tabulated below :—

No. of Expt.	*Medium.*	*Temp.*	*Time.*	*Subsequent Treatment.*	*Result.*
1 (A)	Full digestion	38° C.	18 hrs.	Mounted in dung extract at 25° C. and cooled slowly.	Germination 51 hrs. after expt. began.
2 (B)	"	"	"	"	Preliminary changes.
3 (C)	Dung extract	"	21 hrs.	"	Germination 50½ hrs. after expt. began.
4 (X)	Distilled water	"	40½ hrs.	Mounted in water; treated as above.	No changes.
5 (Y)	Dung extract	"	40½ hrs.	Mounted in extract; treated as above.	Germination after 49½ hrs.
6	$\frac{N}{10}$ Na_2Co_3	"	31 hrs.	Mounted in solution used; treated as above.	Spores distorted.
7	$\frac{N}{100}$ "	"	"	"	Preliminary changes.
8	$\frac{N}{200}$ "	"	"	"	"
9 (I)	$\frac{N}{100}$ "	"	36 hrs.	Mounted in solution at 15° C. and cooled.	Germination after 133 hrs.
10 (II)	" "	"	"	Mounted in solution at 25° C. and cooled.	Germination after 181½ hrs.
11 (III)	$\frac{N}{200}$ "	"	"	"	Preliminary changes
12 (IV)	Dung extract	"	"	"	"

It is noticeable that, treated with the digestive fluids or with dung extract, the spores germinated in every case approximately fifty hours after the beginning of the experiment, and it is probable that they do so in nature about two days after being swallowed by the animal. Germination would appear to be brought about by the combined influence of warmth and an alkaline medium, the action of which, in part at least, is to cause softening of the wall of the spore.

Vegetative Mycelium.

The cells of the mycelium are multinucleate (Fig. 3) and show numerous conspicuous granules, often aggregated on the transverse walls, and corresponding, no doubt, to those observed in several other members of the Pezizineae. No organs of reproduction other than the ascocarps were observed in this form.

Development of the Apothecium.

The archicarp[1] arises as a side branch from a vegetative hypha (Fig. 4), and becomes divided into two, then three (Fig. 5), and finally five or more cells. The terminal cell or ascogonium is oval in shape and larger than the others, and, as development proceeds, it becomes filled with dense contents. In cut and stained preparations it is seen to contain numerous nuclei; the cytoplasm is finely granular and shows a number of small, rounded vacuoles (Fig. 6).

Hyphae grow out from the lower cells of the archicarp and from the branch which bears it, and form a dense weft, above which the ascogonium rises.

So far the archicarp resembles that described by Woronin (22) for *Lachnea scutellata*, Gill. (= *Peziza scutellata*, L.), where a large, oval ascogonium, with granular, somewhat vacuolate contents, terminates the usually three-celled archicarp. Branches, one of which Woronin thinks may be the antheridium, grow up from the lower cells and cover in the, as yet, unbranched ascogonium. Owing to the density of the investing hyphae further development could not be traced.

In *Lachnea stercorea*, however, the ascogonium, before it is surrounded by hyphae, itself sends out a stout branch (Fig. 7), into which several nuclei pass (Fig. 8). This branch is cut off from the ascogonium by a wall on which granules may often be observed. It grows in length and becomes divided, first into two nearly equal cells, and eventually into four, five, or six cells (Fig. 9). Development probably takes place with some rapidity, for, though the unicellular stage was often found, the stage with two cells was only twice observed.

When fully developed this branch consists, as a rule, of five or six cells; those towards the archicarp are narrow, and, though always multinucleate, contain few nuclei. The terminal cell is considerably larger and its nuclei are much more numerous (Figs. 9, 10). In the early, unicellular stage the nuclei of the branch are quite similar to those of the ascogonium,

[1] This term is used for the whole fertile branch other than the antheridium, while the term *ascogonium* is restricted to the reproductive cell or cells containing female nuclei (Blackman and Fraser (4)).

from which it arises, but, as development proceeds, and the ascogonial nuclei increase in size and distinctness, no corresponding change is recognizable in those of the branch ; indeed, after a time, the nuclei of its lower cells are difficult to distinguish from granules.

This branch is not a precocious ascogenous hypha, for it never branches or develops further, and whereas the ascogenous hyphae become emptied as development proceeds, it retains its contents which degenerate *in situ*. Neither does there seem any reason to suppose it of use in conveying food-material to the ascogonium. Although multicellular, it corresponds in other features to the trichogyne of *Pyronema confluens*, and, since it shows evidence of a similar function, it may be conveniently designated by the same name.

Trichogyne. The position of the trichogyne in *Lachnea stercorea* is subject to a considerable amount of variation, sometimes, as in Figs. 9 and 13, it is almost terminal, but more often it is lateral (Figs. 12, 19), or is developed quite near the base of the ascogonium, and passes down almost parallel to the lower part of the archicarp (Fig. 10).

In some cases (Fig. 9) the trichogyne is fully developed, while the ascogonium is still perfectly free, and the sheath has only enveloped the lower cells of the archicarp; in others, however, where development seems scarcely more advanced, the sheath has already grown up about the ascogonium and trichogyne, though the tip of the latter frequently remains uncovered for a time (Fig. 10).

Antheridium. A considerable amount of material was examined in the hope of finding an antheridium arising, as in *Pyronema*, independently of the archicarp, but no structure approaching or becoming incorporated with the young ascocarp from without was ever observed, either in uncut material or in sections.

Often, however, at a stage when the sheath had completely surrounded the archicarp, a large, more or less empty sac was seen to be continuous with the terminal cell of the trichogyne, which at the same time was, in many cases, found to be densely packed with nuclei (Fig. 12).

This sac, or antheridium, is not always well developed, and it was very seldom that a longitudinal section of a clearly defined example was obtained. One such, however, is shown in Fig. 13, where it is seen to be definitely continuous with the short trichogyne.

From such preparations it would appear that the antheridium is a unicellular structure, resembling in this respect that of *Pyronema confluens*, and both here and in oblique and transverse sections it can often be traced, more or less definitely towards the basal cell, or cell of the archicarp next below the ascogonium.

This cell, in *L. stercorea*, as in *Humaria granulata*, differs somewhat from the lower cells of the archicarp. It has denser contents (Figs. 10,

12, 13, &c.), and its nuclei are intermediate in appearance between those of the ascogonium and of the ordinary gametophytic cells (Fig. 14). Such facts are suggestive of some special activity, and it is possibly significant, in this connexion, that the cell next below the ascogonium in *Pyronema confluens*, where the antheridium develops independently, differs in no way from the other supporting cells of the archicarp.

The antheridium contains a number of nuclei, but these either remain *in situ* (Fig. 13) or pass into the terminal cell of the trichogyne (Fig. 12), and they may be observed, often degenerating, but sometimes in an apparently healthy state, late in the development of the ascocarp. This is the case in the specimen shown in Fig. 18, where the ascogonium is already almost empty. The limits of the antheridium and terminal cell of the trichogyne are here difficult to determine.

Often no clear indication of an antheridium could be found, and it would seem that, in some cases, an antheridium is not developed; but it is impossible to speak with certainty of an organ which is distinguished mainly by its shape and position, and which can be studied only in section.

Fertilization. In the specimen represented in Fig. 10 the ascogonium is already giving off ascogenous hyphae, while the trichogyne is still exposed at the exterior of the sheath and no trace of an antheridium can be made out. Again, Fig. 13 shows a branching ascogonium, and a well-defined antheridium, in which, however, numerous nuclei may, at this late stage, be observed; and in Figs. 12 and 18, although many male nuclei appear to have passed into the terminal cell of the trichogyne, they are cut off by its lower cells from the ascogonium.

Such cases are of frequent occurrence, and it would seem, therefore, that ordinary fertilization of the ascogonial by the antheridial nuclei does not normally occur. It is of course possible that this process may still occasionally take place, but among the nearly 300 ascogonia examined no satisfactory evidence of such a fertilization was found.

Another process, however, was frequently observed; the ascogonial, or female nuclei, were seen to fuse in pairs (Figs. 15, 16, 17) in a manner similar to that observed in *Humaria granulata*. The nuclei first fuse (Fig. 15), and the fusion of the nucleoli takes place later (Fig. 16).

The fusions occur at very various stages in the development of the ascocarp; the earliest seen (Fig. 17) was in the ascogonium shown in Fig. 9. In this case it is scarcely possible that antheridial nuclei have entered the ascogonium, and that the nuclei in process of fusion are male and female, for the trichogyne can be traced with great distinctness, and is entirely free either from antheridial or from sheath cells.

Fusions may also be observed when the ascogonium is nearly empty, but the majority probably take place at about the stage represented in Figs. 12 or 13, and they occur in various parts of the ascogonium.

In these particulars *Lachnea stercorea* resembles *Humaria*, whereas in *Pyronema* the fusions all take place at about the same time, and the paired nuclei are aggregated in the centre of the ascogonium. This aggregation is, no doubt, as Harper (16) suggests, a provision for the pairing of the male and female nuclei with the greatest certainty and dispatch, and it is obviously unnecessary in *Humaria*, where all the nuclei are presumably of equal value. The method of fusion in *L. stercorea*, therefore, may be regarded as additional evidence of the reduced nature of fertilization in this fungus.

The early contact stages of fusion cannot be recognized with certainty, but, apart from these, more than eighteen nuclear fusions were observed.

The number of nuclei in the ascogonium is very considerable, and seems to increase with the increasing size of that organ. In order to obtain some idea of the number of nuclei at various stages, the nuclei were counted, in a few cases in a series of sections of an ascogonium or trichogyne, with the following results:—

Young uncovered ascogonium, 267.
Ascogonium, branching just begun, 536.
Unicellular trichogyne, 37.
Trichogyne of Fig. 9.
Terminal cell, 43; lower cells, 20.
Trichogyne nearly covered by sheath.
Terminal cell, 36; lower cells, 25.
Trichogyne of Fig. 12.
Terminal cell, 86; lower cells, 40.

The vacuolate antheridium, continuous with the trichogyne shown in Fig. 12, contained another twenty nuclei, and probably several of the closely packed nuclei in the terminal cell are also antheridial.

The above numbers are of course only roughly approximate, especially as the older ascogonia must contain several fusion nuclei; but it is evident from them that the nuclei of the ascogonium divide. Material was therefore fixed, at various hours of the day and night, in the hopes of finding such divisions. In a few cases indications of a spindle were seen, but the object was too small and too indistinct to be considered conclusive.

Sporophyte. Soon after the sheathing hyphae have grown up about the ascogonium branching begins. The ascogonial branches are of considerable size (Fig. 14) and often grow out freely beyond the investing cells. They contain numerous large, well-defined nuclei, the product, no doubt, of the fusions in the ascogonium.

Eventually the ascogonium becomes emptied of its contents, though a little cytoplasm and a few nuclei may be observed at quite a late stage (Fig. 19). The lower parts of the ascogenous hyphae also become emptied, and by the time that the spores are formed, both they and the ascogonium have disappeared. The contents of the trichogyne, as development pro-

ceeds, become blackened and disintegrated (Fig. 19), and finally it also is lost. At a much earlier stage the antheridium has been obliterated.

The asci develop at the ends of the ascogenous hyphae by the peculiar process of bending over (Figs. 20, 21) observed by Dangeard (7), Harper (15), and others. In the penultimate cell two nuclei fuse (Fig. 22) to form the nucleus of the growing ascus.

The further development of the asci was not specially studied as the object did not appear favourable. Their divisions show an intra-nuclear spindle, and finally eight spores are formed in each.

The peridium never closes completely, as in *Humaria*, or *Ascobolus*, across the top of the ascocarp, but forms a cup-shaped investment. Within is a tangle of vegetative hyphae, amongst which the branches of the ascogonium ramify, and from which the paraphyses eventually grow up. With regard to this character the position of *L. stercorea* is intermediate between that of the Pyronemaceae and such forms as *Humaria granulata*. As in other members of the Pezizineae, a secondary mycelium grows downwards from the ascocarp and absorbs nourishment from the substratum.

General Conclusions.

It seems clear that *Lachnea stercorea* adds another to the list of sexually reduced Ascomycetes. Further, it cannot be regarded as parthenogenetic in the fullest sense, since the male gamete is replaced, as in *Humaria granulata* (Blackman and Fraser (4)), and in certain of the Uredineae (Christman (5), Blackman and Fraser (3)), by a female nucleus.

Thus, with regard to its sexuality, *Lachnea stercorea*, in which the antheridium and trichogyne are present, though not functional, stands in a position intermediate between *Pyronema confluens* (Harper (17)) on the one hand and *Humaria granulata* on the other; and it differs very little from such 'parthenogenetic' specimens of *Pyronema* as were obtained in artificial culture by Van Tieghem (21) and by Dangeard (7). It is, indeed, as has been elsewhere suggested (Blackman and Fraser (4)), not improbable that further investigation may show that, in such cases also, the functionless male nuclei are replaced by female.

In *Humaria granulata* reduction has progressed a stage further; of the trichogyne, if such existed, there is no longer evidence, and the antheridium is also lost. In other particulars the structure and development of the archicarp, and indeed of the whole fruit, closely resemble those of *L. stercorea*.

A trichogyne is formed in a considerable number of the Ascomycetes hitherto investigated.

It occurs in perhaps its simplest form in *Boudiera* (or *Ascodesmis*) (Claussen (6)), where it appears as a small upper portion cut off from the ascogonium, and it seems probable that the terminal cell of the coiled and septate archicarp in such forms as *Aspergillus* (De Bary (11)), *Chaetomium* (Oltmanns (19)), or *Hypocopra* (Nichols (18)), has or had a similar function.

In *Pyronema* the trichogyne, though unicellular, is better developed, and arises as a papilla from the growing ascogonium.

In *Polystigma* (Frank (13), Fisch (12)), in *Gnomonia* (Frank (14)) and in *Collema* (Baur (2)), *Physcia* (Darbishire (9)), and other Lichens, the trichogyne is multicellular, and this is also the case in *Poronia punctata* (Dawson (10)), where, however, the organ is not functional and degenerates early.

In these forms the terminal or receptive cell is enlarged, and to it the spermatia adhere. *Polystigma* and *Gnomonia* have not been found cytologically studied, but in the Lichens the cells of the trichogyne have been found to be uninucleate and connected together by broad cytoplasmic strands.

In the Laboulbeniaceae (Thaxter (20)), both unicellular and multicellular trichogynes occur, but these form a fairly complete series among themselves.

It will be seen that the trichogyne of *Lachnea stercorea*, consisting as it does of a series of coenocytic cells, the terminal of which is considerably larger than the others, is intermediate in structure between the trichogyne of *Pyronema* on the one hand, and that of such forms as *Physcia* and *Collema* on the other. It differs also from the latter in the fact that its constituent cells are not visibly connected by strands of cytoplasm; such connexions may or may not have existed when the organ was functional.

L. stercorea, in the general structure of its ascocarp, in the organization of its large coenocytic ascogonium, and in the character of its male organ, resembles *Pyronema* much more closely than it does any of the Pyrenomycetes. But if *L. stercorea* be supposed to have arisen from such a form as *Pyronema*, it is difficult to imagine to what conditions the septation of the trichogyne was a response.

It may be that the close relation of the ascogonium and antheridium in *L. stercorea* was a late modification, and the multicellular structure of the trichogyne may have given it greater stability in reaching out towards a male organ developed at a distance. In *Pyronema*, however, the antheridium arises separately from the archicarp, and yet a unicellular trichogyne serves to connect the two.

On the other hand it has been held by Thaxter (20) and others that the Ascomycetes may be derived from the Red Algae, through some such

forms as the Laboulbeniaceae, and that the Discomycetes may have been thence developed through Pyrenomycetous ancestors.

The multicellular ascogonium of *Physcia* has been found by Darbishire (9) to be practically a coenogamete, its uninucleate cells being connected by broad strands of protoplasm. In *Ascobolus* also, where the individual cells of the ascogonium are multinucleate, the walls between them are incompletely formed (Harper (16), and in *Boudiera* (Claussen (6)) they do not develop until after fertilization.

A series might thus be outlined, passing to *Lachnea stercorea*, where the walls of the ascogonium are not developed, and thence to *Pyronema*, where the now useless septa of the trichogyne are also lost. *Humaria granulata* would represent a side branch, passing out from the neighbourhood of *Lachnea stercorea*, possibly through *L. scutellata* (Woronin (22)).

A serious difficulty, however, in the way of any such series, whether it pass from the Pyronemaceae to the Laboulbeniaceae, or in the opposite direction, is the structure of the male organs. It may indeed be possible to relate the female organs of the group, but it is far more difficult, in the present state of our knowledge, to derive the spermogonium from the antheridium or vice versa, or to imagine stages by which the one may have replaced the other.

Further, it is of course perfectly possible that the multicellular trichogyne may have arisen separately in different groups of the Ascomycetes in response to similar needs.

SUMMARY.

1. The archicarp of *Lachnea stercorea* consists of several cells and terminates in a large, multicellular ascogonium.

2. From the ascogonium a trichogyne, which is at first unicellular, but eventually consists of four, five, or six coenocytic cells, grows out. *Its* terminal cell is much larger than the others and may become continuous with the antheridium.

3. The antheridium, which is not always fully developed, is a unicellular coenocytic sac; its origin could not be traced with certainty.

4. The male nuclei do not reach the ascogonium, but fertilization of a reduced type occurs, the female nuclei fusing in pairs.

5. Ascogenous hyphae, into which the fused nuclei pass, grow out from the ascogonium, and asci are formed, by the usual method, at their tips.

6. *Lachnea stercorea* is intermediate, with regard to its sexuality, between *Pyronema confluens* on the one hand and *Humaria granulata* on the other, and with regard to the organization of its trichogyne, between *Pyronema* and certain of the Pyrenomycetes.

October, 1906.

LIST OF PAPERS.

1. BARKER, B. T. P.: The Morphology and Development of the Ascocarp in *Monascus*. Ann. Bot., xvii, 1903, p. 167.
2. BAUR, E.: Zur Frage nach der Sexualität der Collemaceen. Ber. d. Deutsch. Bot. Gesellsch., xvi, 1898, p. 363.
3. BLACKMAN, V. H., and FRASER, H. C. I.: Further Studies on the Sexuality of the Uredineae. Ann. Bot., xx, 1906, p. 35.
4. ———————: On the Sexuality and Development of the Ascocarp in *Humaria granulata*, Quel. Proc. Roy. Soc., Ser. B, lxxvii, 1906, p. 354.
5. CHRISTMAN, A. H.: Sexual Reproduction of the Rusts. Bot. Gaz., xxxix, 1905, p. 267.
6. CLAUSSEN, P.: Zur Entwickelungsgeschichte der Ascomyceten. *Boudiera*. Bot. Zeit., lxiii, 1905, p. 1.
7. DANGEARD, P. A.: Sur le *Pyronema confluens*, p. 30, and Apropos d'une lettre du Professeur Harper relative aux fusions nucléaires, p. 46. Le Botaniste, 4me sér., 1894, p. 21.
8. ————————: Recherches sur le développement du Périthèce chez les Ascomycètes. Le Botaniste, 9me sér., 1904, p. 59.
9. DARBISHIRE, O. V.: Ueber die Apothecienentwickelung der Flechte *Physcia pulverulenta* (Schreb.), Nyl. Jahrb. f. wiss. Bot., xxxiv, 1899, p. 329.
10. DAWSON, M.: On the Biology of *Poronia punctata* (4). Ann. Bot., xiv, 1900, p. 245.
11. DE BARY, A.: Vergleichende Morphologie und Biologie der Pilze. Leipzig, 1884.
12. FISCH, C.: Beiträge zur Entwickelungsgeschichte einiger Ascomyceten. Bot. Zeit., Jahrg. 40, 1882, p. 850.
13. FRANK, B.: Ueber einige neue und weniger bekannte Pflanzenkrankheiten. Ber. d. Deutsch. Bot. Gesellsch., Bd. I, 1883, p. 58.
14. ————: Ueber *Gnomonia erythrostoma*, die Ursache, etc. Ber. d. Deutsch. Bot. Gesellsch., Bd. IV, 1886, p. 200.
15. HARPER, R. A.: Zur Kentniss der Kerntheilung und Sporenbildung im Ascus. Ber. d. Deutsch. Bot. Gesellsch., Bd. xiii, 1895, p. 67.
16. ————————: Ueber das Verhalten der Kerne bei der Fruchtentwickelung einiger Ascomyceten. Jahrb. f. wiss. Bot., xxix, 1896, p. 655.
17. ————————: Sexual Reproduction in *Pyronema confluens*, &c. Ann. Bot., xiv, 1900, p. 321.
18. NICHOLS, M. A.: The Morphology and Development of certain Pyrenomycetous Fungi. Bot. Gaz., xxii, 1896, p. 301.
19. OLTMANNS, F.: Ueber die Entwickelung der Perithecien in der Gattung *Chaetomium*. Bot. Zeit., xlv, 1887, p. 192.
20. THAXTER, R.: Contributions towards a Monograph of the Laboulbeniaceae. Mem. Am. Acad. Arts and Sciences, xii, No. 3.
21. VAN TIEGHEM, PH.: Culture et développement du *Pyronema confluens*. Bull. de la Soc. Bot. de France, xxxi, 1884, p. 355.
22. WORONIN, M.: Zur Entwickelungsgeschichte des *Ascobolus pulcherrimus*. Beitr. zur Morph. und Physiol. der Pilze, Zweite Reihe, 1866, p. 1.

EXPLANATION OF FIGURES IN PLATES XXIX AND XXX.

Illustrating Miss Fraser's paper on *Lachnea stercorea.*

Fig. 1. (*a*) Fresh spore; (*b*) spore after treatment with dung extract at 38° C.; (*c*) spore germinating in dung extract. × 500.

Fig. 2. Spore, treated with Na_2CO_3, germinating in ascus. × 500.

Fig. 3. Portion of mycelial hypha, showing nuclei and granules on transverse wall. × 1300.

Fig. 4. Very young archicarp; fresh preparation. × 1000.

Fig. 5. Rather older archicarp; fresh preparation. × 500.

Fig. 6. Section of older archicarp before formation of trichogyne. × 600.

Fig. 7. Archicarp with developing trichogyne. × 600.

Fig. 8. Section of rather older archicarp, trichogyne still unicellular, but cut off from the ascogonium by a wall. × 1000.

Fig. 9. Section of uncovered ascogonium with fully developed trichogyne. × 1000.

Fig. 10. Longitudinal section of young ascocarp; ascogonium just beginning to branch, and trichogyne still exposed at exterior. × 600.

Fig. 11. Transverse section of young ascocarp, showing branching ascogonium, and antheridium continuous with terminal cell of trichogyne. × 600.

Fig. 12. Longitudinal section of young ascocarp, antheridium cut transversely and continuous with terminal cell of trichogyne, into which most of its nuclei have passed. × 600.

Fig. 13. Section of young ascocarp showing antheridium and archicarp cut longitudinally. The ascogonium is branching, and the antheridium is continuous with the terminal cell of the trichogyne, but contains degenerating nuclei. × 600.

Fig. 14. Section showing parts of an ascogenous hypha, ascogonium and basal cell, and some vegetative cells. × 1300.

Fig. 15. Group of ascogonial nuclei, with a large fusion nucleus showing two nucleoli. × 2700.

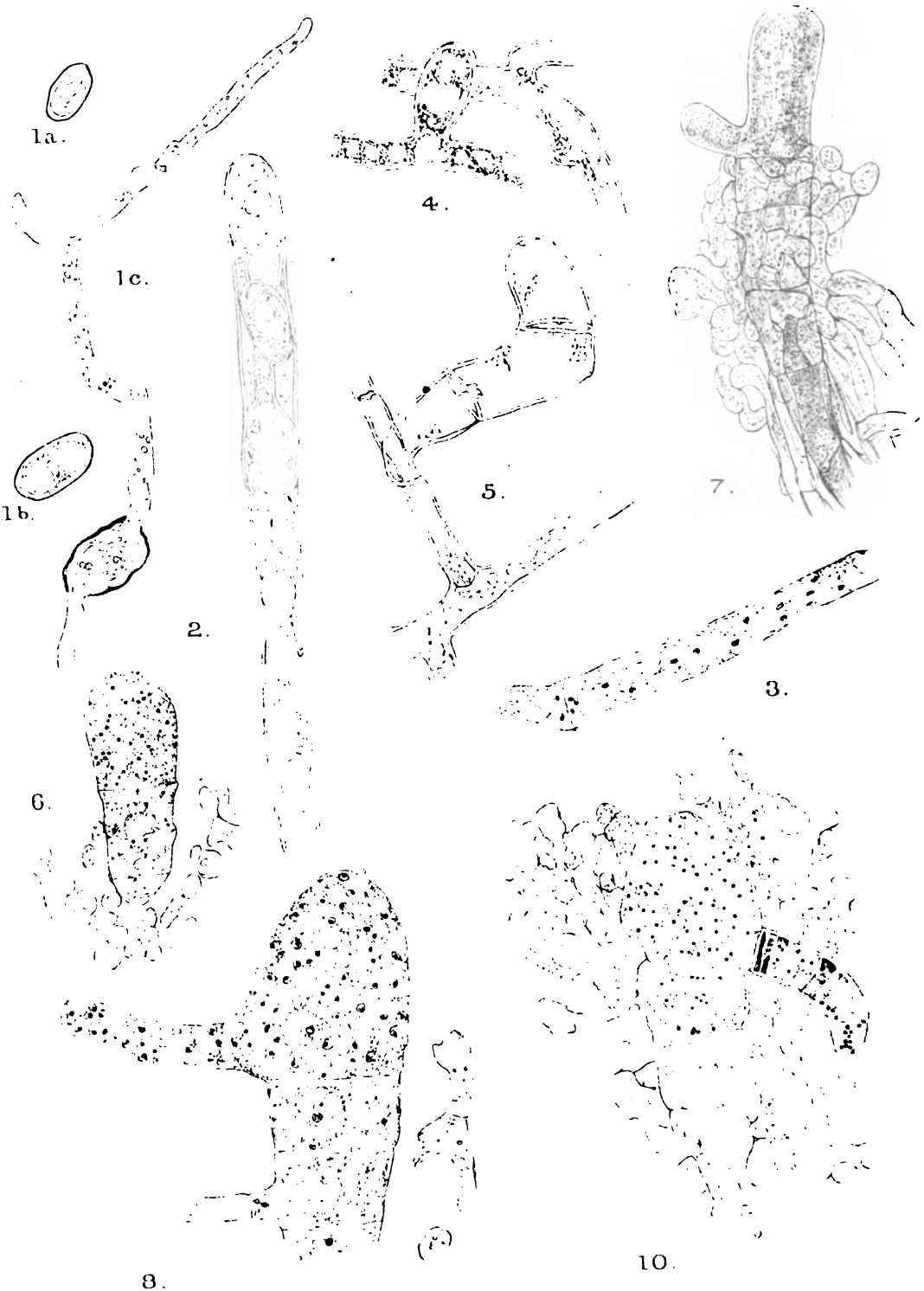
Fig. 16. Fusion nucleus, showing nucleoli in process of fusion. × 2700.

Fig. 17. Group of nuclei from a young ascogonium (Fig. 9), showing fusion of nucleoli. × 2700.

Fig. 18. Trichogyne attached to a nearly empty ascogonium; its terminal cells continuous with an antheridium, and both contain nuclei. × 1000.

Fig. 19. Section through ascocarp at time of fusion in ascus; the archicarp is still visible, the ascogonium is nearly empty, as are the lower parts of the ascogenous hyphae, the contents of the trichogyne are blackened. × 300.

Figs. 20–22. Stages in development of asci at ends of ascogenous hyphae. × 1300.


1a.
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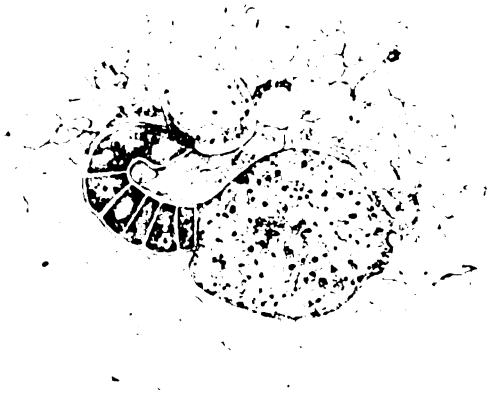

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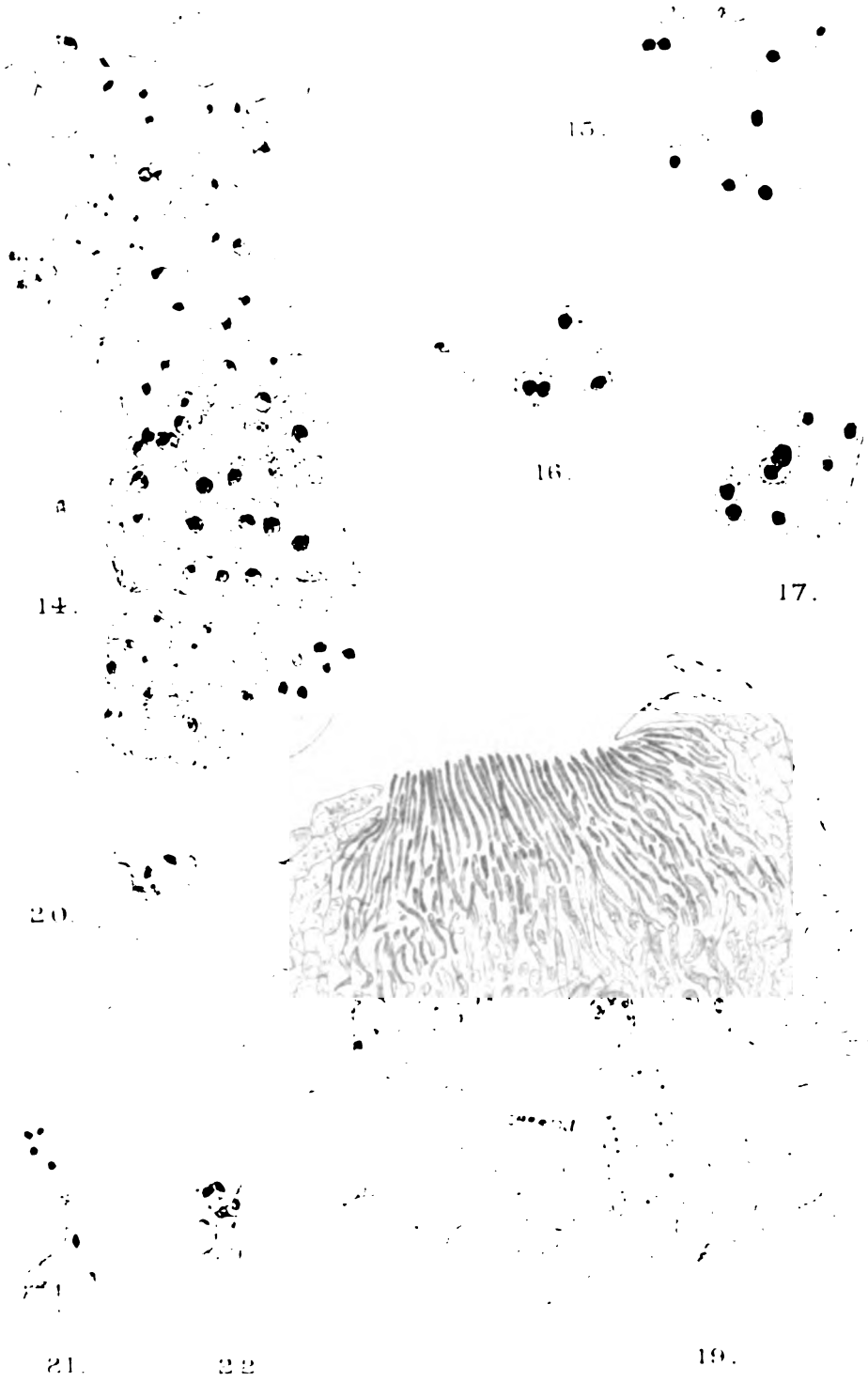

FRASER —— LACHNEA

Secondary Thickening in Kendrickia Walkeri, Hook. f.

BY

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With Plate XXXI.

IT was suggested to me by Professor Bower that the secondary thickening in the stem of *Kendrickia Walkeri* might afford an interesting field for investigation. For the material on which my observations were made, and for much valuable assistance during the course of the investigation, I am indebted to Professor Bower.

Kendrickia Walkeri, Hook. f., a member of the family of Melastomaceae, is an epiphytic climbing shrub, which, like the ivy, climbs by adventitious roots. It is found growing on the Anamallay Hills, S. India, and in Ceylon 'in the forests of the lower Montane zone, and extending down to 2,000 ft. in the Kukul Korale.'[1] The lower parts of the stems are said to be flattened with distichous leaves, but at the summit they are spreading, pendent, bearing leaves on all sides.[2]

Upon preparing sections of the stem, it was found that even the finest twigs showed a slight development of secondary thickening, which, to all appearance, had taken place in a perfectly normal manner.

The arrangement of tissues in this early stage corresponds with the type (*c*) described by De Bary.[3] Here the vascular bundles form a continuous ring, which 'assumes the structure of a vascular bundle', no primary medullary rays being present. Other members of the Melastomaceae also possess this same arrangement. Pl. XXXI, Fig. 1, represents a transverse section of a sector of a stem at this stage. In the centre of the stem is the somewhat large pith, containing an occasional ill-developed cauline bundle, which is not shown in the figure, and, projecting into the pith, are a number of prominent, completely separated, internal phloem-groups. A complete cambium ring is present with secondary xylem to its inner

[1] H. Trimen, Handbook of Flora of Ceylon, vol. xi, p. 200.
[2] Hooker, Flora British India, vol. ii, p. 526.
[3] De Bary, Comp. Anat., Engl. ed., p. 457.

[Annals of Botany, Vol. XXI. No. LXXXIII. July, 1907.]

side, and secondary phloem to its outer side. The xylem is composed mainly of short, square-ended tracheids, with but few vessels. Many of these xylem-cells present rather a curious appearance, due to the shrinking and curling up in the cell-cavity of the separated inner layer of the thickened cell-wall. This layer is presumably cellulose, from the fact that it stains blue with Schulze's Solution. The xylem of *Medinilla*, another member of the Melastomaceae, also shows this same feature. At this stage neither primary nor secondary medullary rays, nor even isolated groups of parenchyma cells, are to be seen.

In slightly older stems, small patches of thin-walled parenchyma cells, with large intercellular spaces, are found situated at irregular intervals throughout the xylem-ring. These patches are, in all probability, equivalent to medullary spots.[1] They are developed from the inner side of the cambium ring, and consist of cells, identical in size and form with the xylem-cells, from which they differ solely by the absence of thick walls (Figs. 2, 3, and 4). The large intercellular spaces might be considered as another point of difference, but this is not so, because patches of thick-walled xylem-cells with similar spaces are also met with at irregular intervals in the xylem-ring (Fig. 5). In addition to these patches there are a number of large areas of parenchymatous tissue, forming wedges which project radially inwards; they are opposite the slight indentations of the stem surface, and are seen to have developed from the inner side of the cambium-ring. These wedges are never, in stems of this age, continuous with the parenchyma of the pith, nor do they communicate with the parenchymatous patches. The cambium opposite each wedge is slightly deflected towards the centre, in correspondence with the surface indentation, but it is always external to the area of xylem-parenchyma forming the wedge. These wedges are not merely radial spokes in the xylem, but are continuous as broad plates or wedges throughout a considerable length of the stem. The fact that there is no equivalent development on the phloem side of the cambium seems to differentiate these structures from ordinary, secondary medullary rays. The parenchymatous cells of the wedge are identical in appearance with those of the patches (Figs. 2, 3, and 4). In transverse section, in many instances, the cells have rounded-off edges with intercellular spaces so exceedingly well marked that the whole tissue presents somewhat the appearance of being composed of marbles, arranged in a series of regular rows (Fig. 7). At somewhat rare intervals, one or perhaps a small group of three to four thick-walled xylem-cells may be seen enclosed in the parenchymatous wedge. This in no wise disturbs the regularity of the tissue-arrangement, as the xylem-cell is identical in size with its parenchymatous neighbours. This regularity of arrangement, which is also

[1] De Bary, Comp. Anat., Engl. ed., p. 492.

characteristic of other Melastomaceae,[1] is a most striking feature, both in the parenchyma and in the thick-walled xylem, of the secondary thickening in *Kendrickia.*

In stems of about this same age the formation of tylosis is of exceedingly common occurrence. It takes place not into old or damaged xylem-elements, but through the pit-membranes of normal, often newly formed tracheids and vessels (Fig. 6). Occasionally in stems of this age the tylosed cells may develop into sclerotic cells. Although thickening of the cell-wall, with pit-formation, is of fairly common occurrence in tylosed cells, yet actual development into sclerotic cells is somewhat rare. Haberlandt[2] cites a few cases in which such a development occurs.

The fact that tyloses are thus prominent features in the still comparatively young stem is of considerable interest, when viewed in the light of the changes which take place in the older stems to be described later.

In most instances this wedge-formation goes on coincidently with the rest of the secondary thickening, and no further changes are observable until the stem has gained considerably in thickness. The size of the stem, however, is not always an indication of the extent of the internal changes. Occasionally a stem of small diameter may have a much more complicated internal structure than one of much greater diameter.

It is after the stem has acquired the structure above described that the apparently mature cells of the secondary zone of thickening begin to show a rather abnormal development, which takes origin in one or several cells, at the central margin of the parenchymatous wedges. These cells take upon themselves new growth, and enlarge or dilate laterally and in a central direction. This enlargement is accompanied by cell-division, and the resulting cells then proceed to encroach upon the hard xylem area, and, as is evident from the figures, this may take place in either of two ways. In Fig. 7 the process has been one of penetration of parenchymatous projections, between the contiguous walls of xylem-elements, by means of which adjoining rows of tracheids have been split apart. In this figure it is found by counting from left to right that there are ten rows of parenchymatous cells and ten rows of tracheids. The new growth from the cells of the third row of the wedge, which is shaded in the figure, has penetrated between the third and fourth rows of tracheids, in the first instance to the extent of three cells deep, then, having separated the second and third tracheids of this fourth row, has proceeded to interpolate the product of its division, between the fourth and fifth rows, until there is a direct parenchymatous connexion between the wedge (*a*) and the patch (*b*). In this case tylosis has played no part whatever, and, as is evident from the figure, not a single tracheid is missing.

[1] Voechting, in Hanstein's Botanische Abhandlungen, iii, p. 1, 1875.

[2] Physiologische Pflanzenanatomie, p. 283.

In Fig. 8 the ingrowth has taken place mainly from the cell (*d*), and here the tracheid (*e*) is the only one of its row which has escaped destruction. From comparison with other examples of similar cases, it is highly probable that tylosis into the missing tracheids first took place, and that these tylosed cells, partly by their own expansive force and possibly partly by absorption, caused the complete disappearance of the row of tracheids, of which (*e*) is the sole representative. No trace of the lost tracheids is to be seen, with the exception perhaps of an occasional slightly pronounced thickening of a wall of one of the cells of the new ingrowth. This, however, may also be present, in cases where there is no suspicion of tylosis having occurred.

In Fig. 9 the processes seem to be coexistent. The cell marked (*a*) in the figure has evidently been a tracheid into which tylosis has taken place, and subsequently its contents have overstepped their bounds and penetrated between two adjoining rows of tracheids. In Fig. 10 rather a good example of this process is seen in a somewhat earlier stage. The two cells (*a*) are probably parenchymatous cells; cells (*b*) and (*c*) seem to be tracheids, into which tylosis has taken place, and which have undergone a slight expansion. A small process from cell (*c*) indicates the point at which penetration between the two neighbouring tracheids is about to take place. In Fig. 11, where the process has advanced somewhat farther, a solitary row of tracheids is represented, completely separated by parenchymatous ingrowths from the rows adjoining.

It is rather remarkable that many of these newly formed parenchyma cells develop farther into sclerotic cells, with peculiarly well-marked lines of stratification and pit-formation. As a general rule these sclerotic cells seem to be dotted about haphazard through the new growth; sometimes surrounded by parenchyma-cells, as in Fig. 11, and sometimes inside tracheids or vessels, as in Fig. 13. These cells may possibly be of some use in the splitting process, by acting as blocks, holding apart the separated masses of xylem, and thus assist in relieving the pressure on the ingrowing parenchyma, which must of necessity be very great. This suggestion, however, will not hold good in every instance, as is obvious from the position of many of the cells.

The ingrowth, as a general rule, starts from the two oldest, diametrically opposed wedges, and proceeds towards the pith (Fig. 15), but later the younger, less deep wedges, and even many of the isolated parenchyma patches follow suit.

The result of the process is the division of the axial woody ring into a varying number of small portions, which lie embedded in a mass of large-celled parenchyma (Fig. 14). The arrangement of the separate portions is by no means regular, and varies in different parts of the stem. Occasionally very small fragments of wood, containing only a few tracheids, are found

occupying an oblique position, surrounded by a mass of parenchyma-cells. These have evidently been separated from their neighbours, twisted out of position, surrounded, and then left behind by the cells of the advance guard of the new ingrowth.

The material at my disposal contained only one portion of a comparatively old stem (Fig. 17), but from an examination of this it was quite evident that the changes in *Kendrickia* were by no means at an end when the parenchymatous communications between the wedges and the pith were effected. No formation of secondary cambium in the large-celled parenchyma of the ingrowth was ever observed, but it seemed as if the quiescent cambium, lying between the original internal phloem-groups and the axial woody ring, had, stimulated apparently by the arrival of the cells of the ingrowth, become active once again, and cut off xylem on the one side and phloem on the other. Only four such groups were noted, but from lack of mature material further developments could not be observed.

General Comparisons.

From the naked-eye appearance (Figs. 15, 16, and 17) the stem of *Kendrickia* would seem to resemble rather closely the stem of a climbing *Bignonia*, or one of the Malpighiaceae. In reality, however, the cases are very dissimilar. In these plants, at the stage which would resemble Figs. 15 and 16, the areas of soft tissue, the so-called furrows of Schenck, consist of bast-plates with cambium at their central margins, and result from a decreased formation of secondary xylem, with increased production of secondary phloem at these several points. Very different is it with *Kendrickia*, where the cambium ring remains complete, is never broken up, nor more than slightly deflected towards the pith, and where the appearance of furrowing of the wood is due entirely to the difference in thickening of the cell-wall in adjoining areas of the secondary xylem-elements. The furrow in the one case contains bast, and in the other xylem-parenchyma.

A comparison of the later changes is of great interest. In *Tetrapteris*, one of the Malpighiaceae, the cambium at the inner margin of the furrow, after a period of quiescence, again becomes active, and cuts off xylem and phloem at the sides of the furrow. The medullary rays of the axial woody ring, lying in the prolongation of the furrow, then proceed to stretch and divide, and form broad bands of dilated parenchyma-cells, which split the woody ring into a definite number of segments.[1] A somewhat similar process takes place in the stems of many of the Bignoniaceae.

Hill[2] points out that in the tuberous portions of the roots of *Bignonia*

[1] Schenck, Beiträge z. Biol. u. Anat. d. Lianen, Pt. ii, p. 115.
[2] Ann. Bot., vol. xii (1898), p. 323.

Unguis there is a 'breaking up of the xylem into separate masses, often by a certain amount of dilatation-parenchyma'. Unfortunately he does not state the origin of the dilatation-parenchyma, nor the mode of breaking up of the xylem, so that a further comparison of this root with the *Kendrickia* stem cannot be made.

In the stems described above, the splitting which takes place is not, strictly speaking, an entirely new formation. The medullary rays contained in the axial woody ring connect the furrows with the pith, and therefore from the first actually map out the wood by fine lines of divisions into its various segments. Thus by the dilatation and new growth of apparently mature parenchyma a microscopic line of separation is converted into a large conspicuous split.

In *Kendrickia* the axial woody ring contains no medullary rays, therefore there are no existing lines of separation of which to make use. Yet in both cases, though by widely different methods, the same result is reached, viz. large parenchymatous communications between the internal and the external soft tissues.

Schenck [1] discusses the causation of this new growth, and advances as probable the theory that the continued renewal of the bast-plates causes a tangential or radial tension in the axial woody ring, which acts as a stimulus, leading to a dilatation of the lines of least resistance, viz. the primary medullary rays and the pith. In *Kendrickia,* however, the first evidence of the change about to occur is the dilatation and division of one or more cells of the wedge, therefore there is nothing in the nature of preliminary anatomical changes which could constitute a stimulus, tangential or otherwise.

Farther, in the stems mentioned above, the woody ring is divided into a definite number of segments, each with a distinct portion of pith attached to its inner margin; and immediately succeeding the splitting, isolated secondary cambiums become evident at varying intervals in the dilatation-parenchyma. In *Kendrickia*, on the other hand, the separated portions of wood are so small in many cases that they by no means deserve the name of segments; also the pith, if attached, is not always recognizable as such; and lastly, isolated, secondary cambiums are never developed in the dilatation-parenchyma, even in stems at the age of that in Fig. 17. It is quite possible, however, that they may develop in still more mature stems.

Thus it is seen that, although the splitting of the axial woody ring is by no means a rare phenomenon in plants, yet the details of the process in *Kendrickia* are in some ways quite unique. The part which tylosis plays in the process, by utilizing the space contained in the lumen of the tracheids and vessels, with the subsequent destruction of the identity of these cells,

[1] Loc. cit., p. 231.

together with the separation of the rows of tracheids by the parenchyma-cells cutting a path for themselves, seem to be without parallel in the, as yet, described Anatomy of Plants.

EXPLANATION OF FIGURES IN PLATE XXXI.

Illustrating Miss Clark's paper on *Kendrickia Walkeri*.

Fig. 1. Transverse section of a young stem of *Kendrickia Walkeri*. *M* = pith; *int. ph.* = internal phloem; *xy* = xylem; *c* = cambium; *ext. ph.* = external phloem; *K* = cortex; *P* = commencing periderm formation. × 180.

Fig. 2. Transverse section of a slightly older stem showing commencing wedge formation. × 120.

Fig. 3. Longitudinal radial section at the margin of a wedge. × 120.

Fig. 4. Tangential section of the same. × 120.

Fig. 5. Patch of xylem-cells with large intercellular spaces. × 180.

Fig. 6. Beginning of tylosis. *a* = parenchyma-cell; *b* = vessel. × 180.

Fig. 7. Transverse section showing early stage of splitting of the axial woody ring. *a* = central margin of wedge; *b* = parenchymatous patch. × 120.

Fig. 8. Ditto, at a slightly older stage. *a* = wedge; *b* = patch; *c* = pith; *d* = cell from which the ingrowth has mainly taken place; *e* = only tracheid of a row which has escaped destruction. × 120.

Fig. 9. Ditto, at the margin of the ingrowth. *a* = parenchymatous cell. × 120.

Fig. 10. Ditto. *a-a* = parenchymatous cells; *b* and *c* = tracheids filled with tylosed cells. × 120.

Fig. 11. Ditto, at a fairly old stage. *a* = wedge; *b* = isolated tracheid; *c* = pith; *d* = a split or gap in the tissue; *T* = isolated row of tracheids. × 120.

Fig. 12. Ditto. *a* = wedge; *b* = sclerotic cells; *c* = pith; *d* = vessel filled with tylosed cells. × 120.

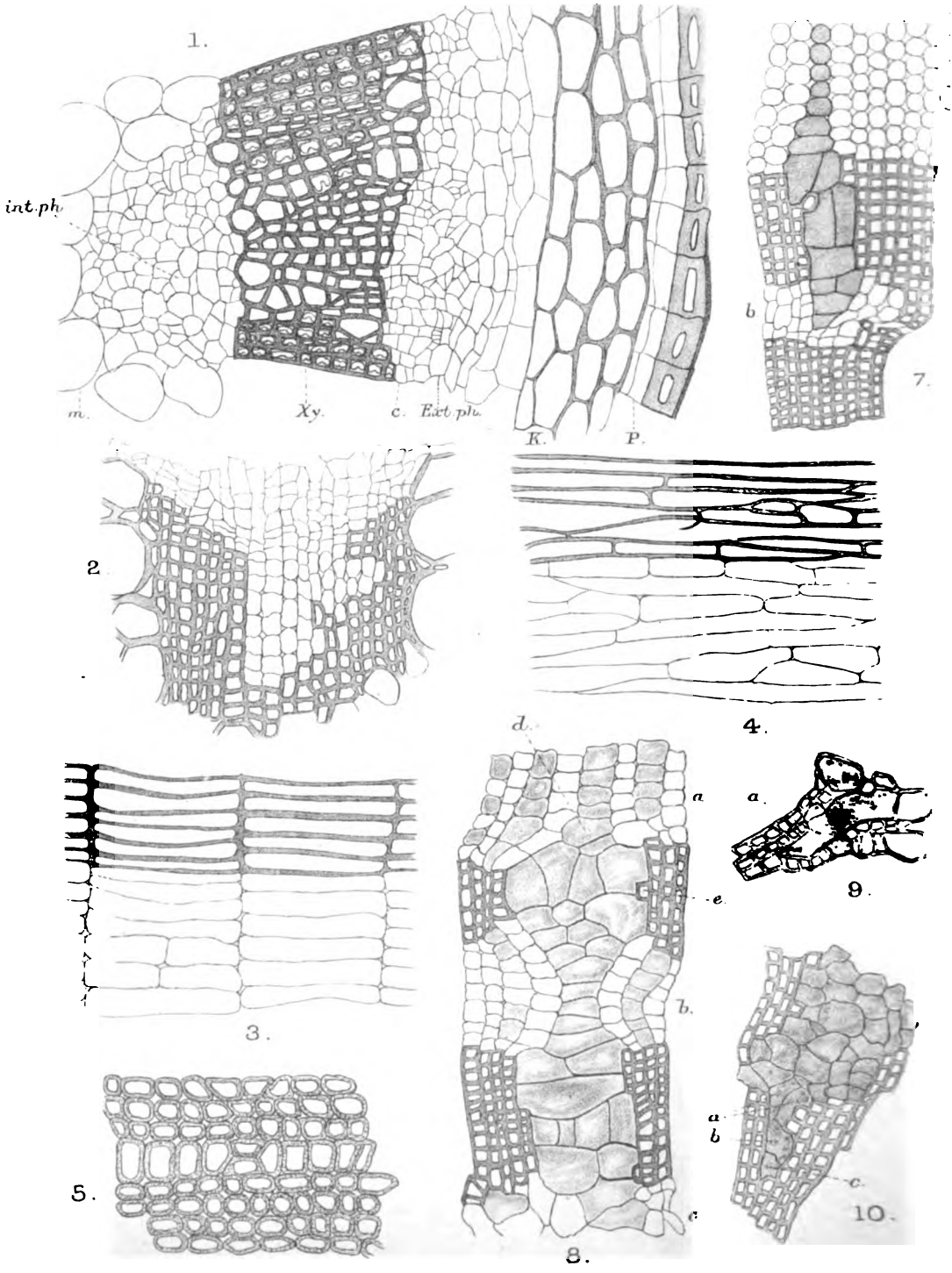
Fig. 13. Longitudinal radial section at the margin of the ingrowth. *a* = cells at the margin of the wedge; *b* = cavity of a vessel; *c* = a tylosed cell which has developed into a sclerotic cell; *d* = a tylosed parenchyma-cell. × 120.

Fig. 14. Transverse section of the old stem showing a separated portion of the axial woody ring embedded in dilatation-parenchyma. The arrow represents the direction taken by the ingrowth. *a* = wedge side; *c* = pith side. × 80.

Fig. 15. Photograph of the cut surface of the stem. The faint shading connecting two of the wedges through the pith indicates the position of the first ingrowth. × ½.

Fig. 16. Ditto. Showing slightly different configuration of the wood. × ½.

Fig. 17. Ditto, of old stem. × ½.



CLARK — KENDRICKIA

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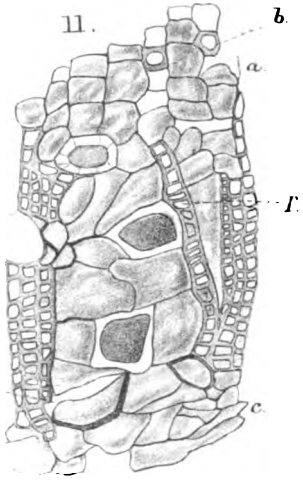

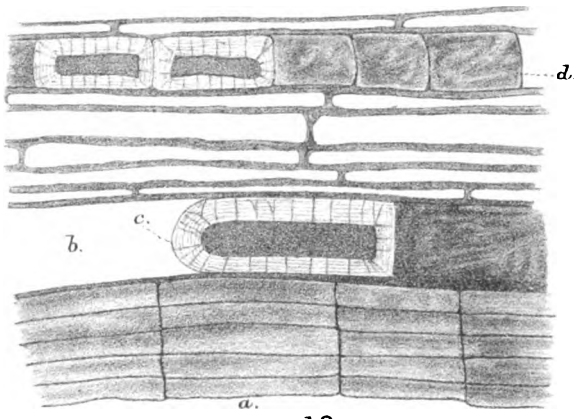

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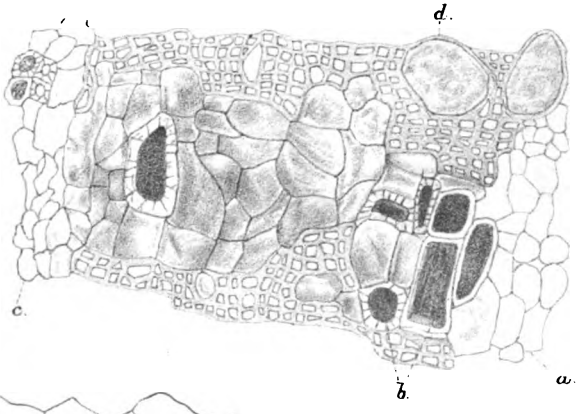

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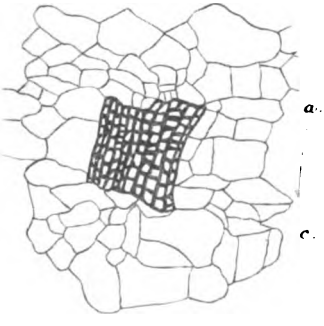

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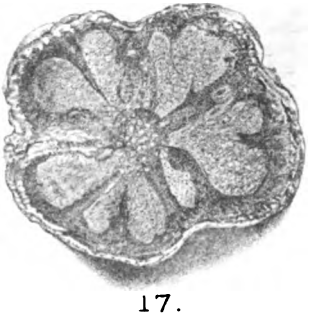

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Huth, lith. et imp.

The Anatomy of Palaeostachya vera.

BY

GEORGE HICKLING, B.Sc.

Assistant Lecturer in Geology in the Victoria University of Manchester.

With Plates XXXII and XXXIII, and four Figures in the Text.

MORE than thirty-seven years ago Williamson described before the Manchester Literary and Philosophical Society[1] 'A New Form of Calamitean Strobilus', from a fragmentary calcified specimen which Mr. Butterworth had found in a nodule from the Upper Foot Coal at Roe Buck, in Strinesdale, Saddleworth. The title of that paper is interesting. It was a 'new' Calamitean cone, because another had already been described by Carruthers and Binney, and the latter cone (*Calamostachys*) was admitted by Williamson to be Calamitean. Indeed, he states (p. 261) regarding the two cones that 'the differences which they present' are 'but generic, not ordinal ones', and, further on, he argues that his new cone must be Calamitean 'since no one appears to doubt that such is the character of Mr. Binney's strobilus'. Early in 1887 more specimens of the new cone were found among nodules from the Oldham district, and were described by Williamson in the same year before the Royal Society[2] as 'The True Fructification of Calamites.' As is well known, the title of this second paper was meant to emphasize Williamson's later conviction, that *Calamostachys* could not be a Calamitean cone. With the true ardour of a man who is sure of his new belief, he tells us 'Mr. Carruthers believed he had found it [the fruit of *Calamites*] in *Calamostachys Binneyana*, and Mr. Binney arrived at a similar conclusion. I have always rejected these conclusions because of the conspicuous differences between the morphology of the Calamitean twig and that of the axis of *Calamostachys*.' As a memorial of this new belief, Seward,[3] when he referred the 'True Fructification' to Weiss's genus *Palaeostachya*, chose '*vera*' as the specific designation.

[1] Williamson, '69. [2] Ibid., '87. [3] Seward, '98, p. 358.

[Annals of Botany, Vol. XXI. No. LXXXIII. July, 1907.]

Latterly, in his joint paper with Dr. Scott,[1] Williamson yielded again to the view, now undoubted, that *Calamostachys* was a true Calamitean fructification, thus admitting it to a closer relationship with *Palaeostachya*.

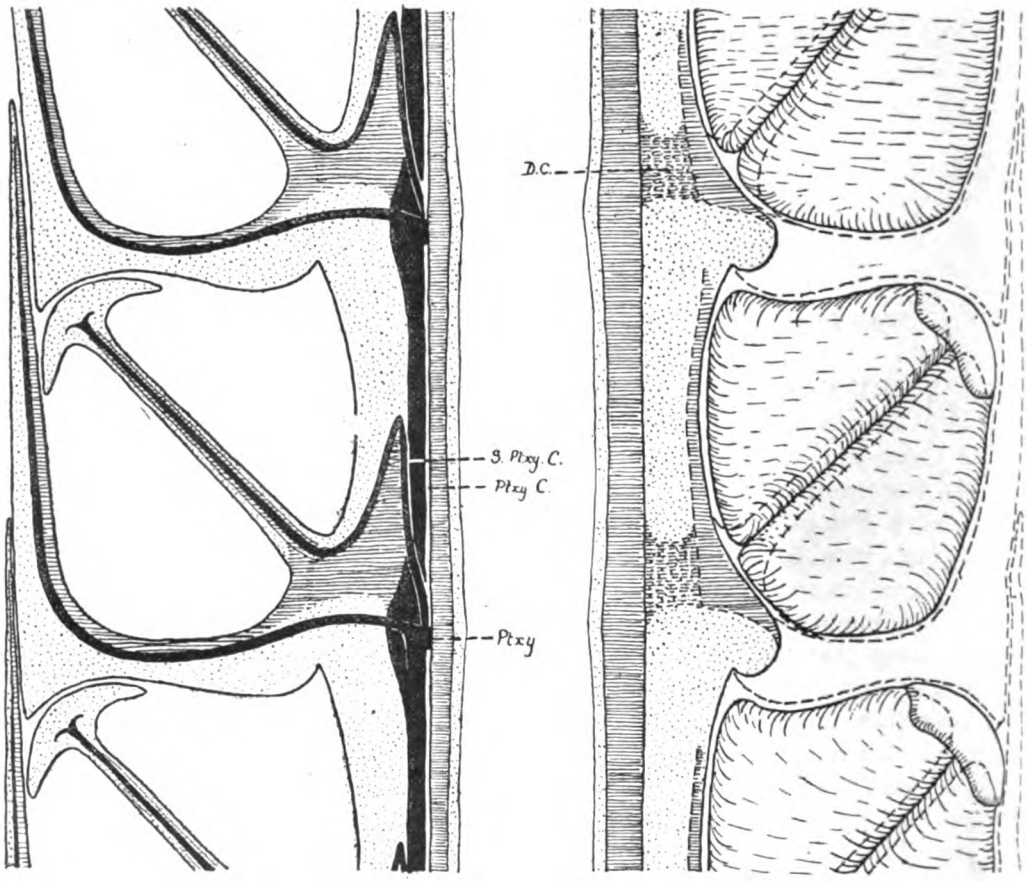


FIG. 1. Diagrammatic Longitudinal Section of Cone. × 14. Vascular tissue, *black*. Soft parenchyma, *dotted*. Sclerized, *lined*. *Left* side passes through bundle; *right*, between bundles. In each case plane of section would pass *between* adjacent sporangia; on left these are omitted for clearness, on right they are inserted as solid objects in surface view. *D.C.*, 'disc-canal.' The sclerized tissue is here indicated (by broken lines) as seen through the parenchyma, which fills it; *Pt.xy. C.*, protoxylem-canal of main bundle; *S. Pt.xy. C.*, protoxylem-canal of sporangiophore-trace; *Pt.xy.*, plug of protoxylem left in the canal at the node. The only fact of importance indicated in the diagram for which the evidence is not quite satisfactory is the superposition of the sporangiophores over the bracts, and the superposition of the successive whorls.

This view of the relationship I hope to confirm in the present paper with the more ample data for comparison, provided by a complete reinvestigation of all the existing sections of *Palaeostachya* undertaken at the suggestion

[1] Williamson and Scott, '94.

of Dr. Scott. This examination has also brought to light certain new features which I think tend to modify, in some considerable degree, our ideas regarding the position occupied by *Palaeostachya* in the Calamarian series of fructifications.

A slightly revised account of the anatomy of this cone was published in the joint paper just referred to,[1] while Dr. Scott summarized his view regarding it, and gave an excellent figure of a transverse section in his collection, in his 'Studies.'[2]

Considerable difficulty has been experienced in preparing a complete account of the anatomy of this cone. The state of preservation is only fair, and the sections themselves being old, are badly cut and thick. Consequently strong and carefully controlled illumination is necessary to make out the details. These difficulties, coupled with rapid working, are probably responsible for the numerous mistakes in Williamson's account.

General Features. I have little doubt that the general external appearance of this cone would be fairly represented by Weiss's figure of his *Palaeostachya pedunculata*.[3] In diameter our cone was about the same as Weiss's specimen. Its length cannot now be determined, but it does not appear to have been short. It was pedunculate, and from the fact that four successive transverse sections passed through one peduncle, we may fairly conclude that that particular peduncle was not less than half an inch in length. The internodes had a length of about 4·7 mm. Round each of the swollen nodes of the axis were placed usually eighteen oblique sporangiophores, in the axils of about the same number of bracts. The bracts were free, but imbricating except towards the tips, first horizontal, then ascending. They probably hid completely the fertile parts from view. The sporangiophores were peltate, and each bore four sporangia, all the sporangia in a whorl being closely appressed. The axis was typically Calamitean in its anatomy, fistular, without nodal diaphragms, but with a complex system of supporting tissue in the cortex at each node. Text-Fig. 1 shows this sclerized tissue in longitudinal section. It formed a thick plate-like ring as described below, with (usually) nine unsclerized intervals, which now appear as 'canals' owing to the non-preservation of the soft parenchyma which formerly filled them. On the right side, the section passes through these canals. The soft tissue in them is imagined to be transparent, and the sclerized part to be seen through it. The eighteen bundles of the axis were approximated in pairs lying between these 'canals,' as seen in Pl. XXXII, Fig. 7. The diagrammatic section shows clearly the anomalous course of the sporangiophore-trace, which is the most remarkable point now brought to light. It indicates also the swelling of the vascular tissue at the nodes by the addition of secondary tracheids.

[1] Williamson and Scott, '94, p. 916. [2] Scott, '00, p. 61.
[3] Weiss, '76, Taf. xv.

As mentioned in the description accompanying the section, the evidence that the sporangiophores were superposed on the bracts, and the members of the successive whorls also superposed, is insufficient (see p. 375).

The Axis. The interest of this structure centres mainly in its very typical Calamitean anatomy. It alone causes *Palaeostachya* to be undisputed as the cone of a Calamite. A transverse section of the peduncle is not to be distinguished from a young vegetative shoot, while a similar internodal section through the fertile portion differs only in the slight

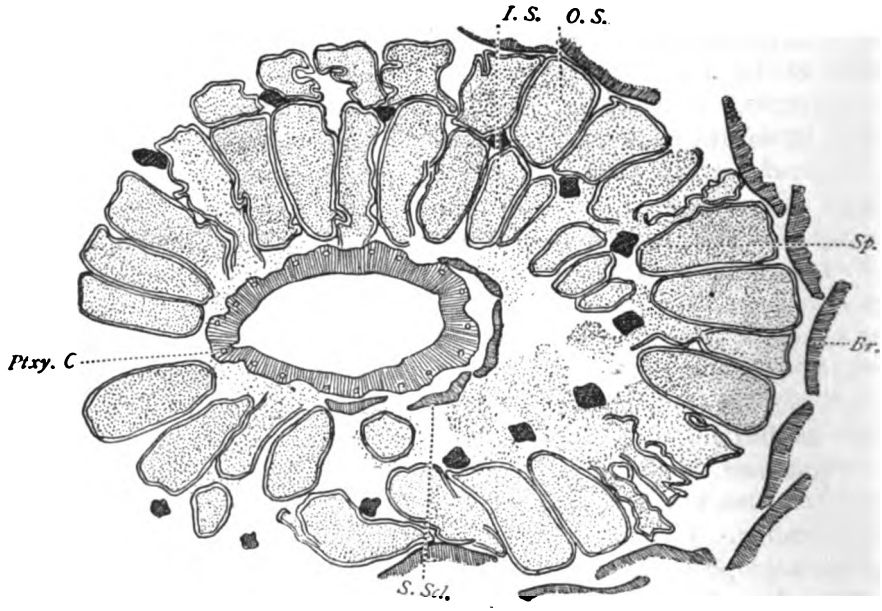


FIG. 2. Slightly oblique internodal transverse section. R. 31 × 9½ dia. Plane of section ascends from right to left. This section has sporangia more complete than any other, and in conjunction with Text-Fig. 1 will give a fairly complete idea of the cone. It should be remembered that the soft cortex is completely removed. *Br.* = bract; *Sp.* = sporangiophore; *O.S.* = outer sporangium; *I.S.* = inner sporangium; *S.Scl.* = sub-epidermal sclerenchyma; *Ptxy.C.* = protoxylem canal.

'pairing' of the bundles. Two further peculiarities distinguish this axis from the vegetative stem; there were no nodal diaphragms across the medullary cavity, while these were functionally replaced by nodal rings of sclerized cortex—the 'nodal discs' of Williamson.

Cortex. No section shows this completely preserved, and only one shows fragments (Fig. 11). In this it appears to extend over about a quarter of the radius of the complete axis. It is a simple, large-celled, parenchymatous cortex, with its largest elements towards the exterior. On the outside of the xylem-bundles, groups of smaller elements may be traced, which no doubt represent the phloem (Fig. 8, *phl.*). The epidermis is preserved only in a section through the insertion of a sporangio-

phore. It has here divided to form four or five layers of periderm cells (Fig. 10, *pd.*). The epidermal layer itself is perfectly normal.

The most interesting feature of the cortex is its elaborate system of sclerized elements. Briefly, the stereome structure consists of a series of thick rings, one at each node, placed like phlanges round the cylinder of xylem strands and sclerized medulla, and braced together externally by vertical sub-epidermal sclerized strands. Such a skeleton must have made the axis extremely rigid, and no doubt points to an erect habit for the cone. The nodal rings (the 'discs' of Williamson) are simply zones of sclerized parenchyma occupying the entire width of the cortex, and having a vertical thickness about equal to their width (Figs. 1, 2, and 7). The junction of the sclerized zone with the soft cortex was not sharply defined, the extent of the sclerization here, as in other parts of the cone, being variable. Since a complete sclerized ring would be a physiological impossibility, large rounded intervals were left, through which the soft tissue was continuous (Figs. 1 and 7, Text-Fig. 1). By the decay of the soft tissue they now appear as 'canals' (the 'cortical lacunae' of Williamson), but in one case (Fig. 8, *par.*) there is sufficient soft tissue left to justify the view here stated. These 'canals' alternate in position with the pairs of bundles, and are usually nine, occasionally eight or ten in number. The 'disc' provided a firm basis for the attachment of both bracts and sporangiophores, the mechanical tissue of each of which joined on to it.

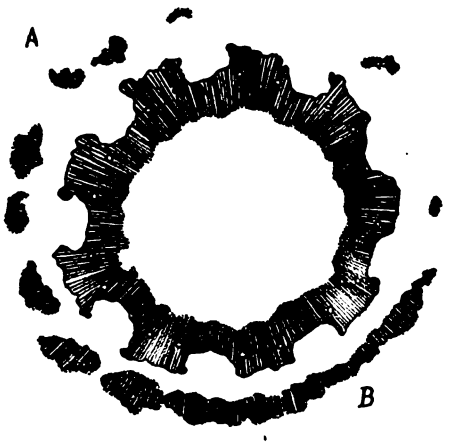


FIG. 3. Oblique-transverse section. Q. 334. × about 11. Showing irregularity of sub-epidermal strands of cortical sclerenchyma. The plane of the section rises from *A* to *B*.

From the edge of the disc, between the bases of the sporangiophores, vertical bands of sub-epidermal sclerization swept upwards. Sometimes the whole band from node to node was sclerized, sometimes the upper portion appears to have remained soft, so that the sclerized part remains as a long, upward-projecting tooth. In some cases these bands joined up laterally just above the disc to form a complete sheath of sclerized hypoderma. It is evident that here, as in the disc itself, the extent of the sclerization is somewhat variable. These variations are indicated in Text-Fig. 1, and in the accompanying Text-Fig. 3.

Medulla. Within the ring of vascular bundles there was a zone of medullary tissue around the pith-cavity some five to ten cells in thickness.

The average size of the cells increases towards the centre, and they are all vertically elongated except at the nodes (Figs. 1, 2, 13). Only two or three layers immediately round the cavity were thin-walled, the remainder sclerized. The medullary rays consisted likewise of regular thick-walled cells. The rays between the bundles of a pair merge insensibly into the medulla internally, and the 'disc' externally. Those between the pairs of bundles stop short at the 'cortical canals.'

Vascular System. The wedge-shaped xylem bundles form a ring round the medulla. Of these bundles there are usually eighteen, occasionally from sixteen to twenty, arranged normally in pairs, which lie alternately with the 'large canals' of the 'nodal discs.' This pairing of the bundles is the most striking feature in the anatomy of the axis. It is perhaps more apparent than real, and seems to be due to the abnormal orientation of the bundles necessitated by the mechanical arrangements at the nodes, where the phloem must be turned so as to lie within the 'canal,' while the xylem must correspondingly rotate in the opposite direction. If the bundles were normally orientated the disc would obviously be much weakened. Thus the pair of bundles lying between two canals have their xylem masses turned towards one another, and so approximated, while the phloem masses are turned away. Each bundle is endarch, and has a '*carinal*' canal representing the degenerated protoxylem exactly as in *Calamites* and *Equisetum*. Traces of the broken tracheids may be seen in the longitudinal section of the canal.

At the node the protoxylem remains, obliterating the canal at that point (Fig. 1, *ptxy.*). In a mid-internodal section, each xylem bundle is seen to consist of some twenty to forty very small tracheids. The whole of this wood appears to be primary and centrifugal, though one section shows one tracheid apparently centripetal, but only, I think, because the section does not cut the carinal canal medianly at that point.

The tracheids are all either spiral or annular. On approaching the node the bundles increase rapidly in size by the addition externally of a considerable mass of secondary wood. As in *Calamites* these secondary tracheids were, in the youngest condition, limited to the nodes only, while the later-formed tracheids extended successively further and further into the internodes. It was this characteristically Calamitean development of secondary xylem which first convinced Williamson of the affinities of this cone. These secondary tracheids are larger than the primary ones, and are scalariform or pitted with two to four rows of eye-shaped pits (Fig. 14, *B*, *C*). Where this secondary wood is thickest, there are about ten to fifteen layers of tracheids (Fig. 3).

The node, using this term to indicate the level at which the vascular bundles give off their branches to the appendages, and at which there is the usual shortening up and disturbance of the course of the

tracheids, was situated just a little below the lower limit of the 'nodal disc.' The secondary wood was here thickest, and gradually died out above and below, extending in the upward direction just about to the upper limit of the 'disc.' I have diligently endeavoured to determine whether or not there is an alternation of bundles in the successive internodes. No tangential section exists sufficiently perfect to determine whether the successive whorls of appendages are superposed or not. From an examination of the numerous sections which cut more or less transversely through the node, I feel little doubt that no regular pectination occurred, while, on the other hand, one or two sections show features which seem explicable only on the assumption that occasional communications (probably irregular) did occur between adjacent bundles.

Vascular Supply of the Appendages. The course of the bundles which supply the bracts can only be traced with great difficulty, but it is evidently quite simple. Several of the bracts themselves show traces of quite a large xylem-bundle, and in the transverse section (Fig. 15) a single median bundle is clearly seen. There is no evidence in any case of more than a single bundle in the bract, though as Fig. 15 represents a section not far from the tip, it cannot be regarded as conclusive. The trace in the cortex is practically always lost, owing to the removal of the surrounding parenchyma, and for the same reason it is generally lost from the bract itself (see below, p. 378). A single section shows very imperfect macerated fragments of this bundle in the cortex, but its origin from the main bundle is fairly well seen in several cases (Fig. 20). It arises from the primary wood of that bundle just where the carinal canal is obliterated, and passes radially outwards and slightly upwards through the nodal secondary wood, which course was no doubt continued through the cortex till it curved downward into the bract. No gap is left in the main bundle, and as all the tracheids throughout the bract-bundle appear to be spiral or annular, it may be concluded that it received no contributions from the secondary wood.

Sporangiophore Bundles. Since the course of these bundles is the main point which fixes the position of this cone in the Calamarian series, it is a fortunate circumstance that they are accompanied through the cortex by sclerized tissue, and so are completely preserved. Yet the totally unexpected nature of the course led to its remaining long undiscovered during the present investigation. The origin of the bundle is *immediately* above that of the bract-bundle; i. e. they originate one above the other at the same node. But the sporangiophore bundle does not separate from the main bundle until it has ascended through almost exactly half the internode. It is then sharply reflexed, descends rapidly to the upper limit of the sclerized 'nodal disc,' and sweeps horizontally across the upper part of this to the base of the sporangiophore. The great

importance of this course, and the interpretation to be placed on it, justifies the further particulars now to be given. The course of the bundle across the upper part of the sclerized disc is clearly seen in radial longitudinal section in Fig. 21. The figure also demonstrates the fact that the bundle approaches the axis in an ascending, not a descending path, and though it is difficult to distinguish tracheids elsewhere, sufficient traces may be found to prove that the bundle followed the outer edge of the triangular patch of sclerenchyma limited internally by the space *S.Ptxy.C.* At the apex of this triangle, traces of reflexed fibres may be seen, as indicated in the drawing. The space *S.Ptxy.C.* I can only interpret as the protoxylem-canal of the ascending limb of the bundle. Any doubt which might be felt as to the bundle really passing round the sclerenchyma is completely removed by the numerous oblique-transverse sections, in which the sclerenchyma is seen forming the angular projections between alternate pairs of the main bundles, and showing the sporangiophore bundles hanging on to the projecting angles (Fig. 7, *Sp. tr.*). It remains to prove that that point where the sporangiophore-trace comes into contact with the main bundle, at the apex of the triangle of sclerenchyma, is not the origin of the trace. The evident reflexion of the accompanying fibres (Fig. 21) and the existence of the canal *S.Ptxy.C.* suggest otherwise. Moreover, were that point a true node, one would expect the ground tissue cells to be 'shortened-up,' and the protoxylem canals of the main bundles to be obliterated as at the ordinary nodes. But no such modifications exist. Finally, the canal I have referred to in Fig. 21 as probably the protoxylem-canal of the trace, is a constant feature in the transverse sections, especially in those through the disc, where it appears as a small lacuna external to the main carinal canal, approaching nearer to the latter canal as the section gets closer to the node, till, immediately above that point, the two spaces become confluent, causing an 8-shaped space (see Figs. 12, 13, and 16). I think this leaves no doubt as to the correctness of the view here taken.

Figs. 6 and 19 show the sporangiophore-trace crossing the 'disc' in transverse section, and give a fair idea of the dimensions of the bundle. In Fig. 6, *M*, a patch of dark tissue is seen at each end of the mass of tracheids. This tissue forms a strand accompanying the bundle, and crossing the 'disc' immediately underneath it, so that in sections at a slightly lower level the separate patches here seen become confluent. These dark patches are very conspicuous features in sections of the 'disc' (Fig. 7, *M*). One such patch is drawn in Fig. 18. The cells are elongated, with square or oblique ends, and are almost completely filled with a dense black material. At first I took this tissue for sclerenchyma, in consequence of its being always well preserved, but it is obviously not ordinary sclerenchyma, and is probably not sclerized at all. The 'melanotic' cells of the

ordinary calamites possibly represent a similar tissue. The continuation of this tissue in the sporangiophore is referred to below.

The Bracts. The number of these appendages in each whorl appears to have been equal to that of the sporangiophores, or nearly so, *certainly* not double that number as formerly stated. The adjoining Text-Fig. 4 is a camera-lucida outline sketch of a section fairly supporting this statement. The crushing of the bracts towards the left may account for their appearing

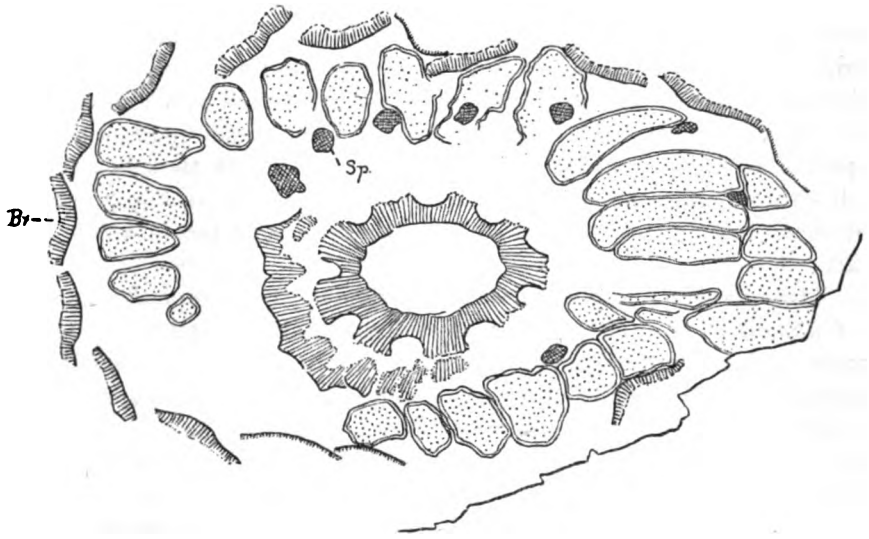


FIG. 4. Oblique-transverse section, W. 1568, illustrating the fact that the number of bracts, *Br.*, is about equal to that of the sporangiophores, *Sp.* There were eighteen sporangiophores (= projections on axis × 2). Camera-lucida outline sketch.

rather more numerous on that side than theory requires. Text-Fig. 2 may also be referred to in this connexion.

Williamson [1] describes the surface of his first specimen of this cone as showing 'a series of strongly marked ridges and furrows, the former being apparently about twenty in number'; and then adds that each furrow appeared to be occupied by one bract. In that cone there were twenty sporangiophores.

The bracts were free, or very slightly connate at the base, first horizontal, then ascending. The horizontal bases were in contact laterally, the ascending portions imbricating, except towards the tip. The general form must have been broadly lanceolate, the widest point being near the base of the ascending portion, while the apex was probably acuminate, and overlapped to a small extent the bracts of the whorl above. The only

[1] Williamson, '69, p. 249.

tissue generally preserved is the sclerenchyma, which extended as a broad band under the ventral surface, and occupying about two-thirds of the width of the bract (Fig. 15, *scl.*). The figure just referred to shows the lozenge-shaped form of the complete bract. The section is probably from about the middle of the ascending portion. The epidermis is well marked. Towards the dorsal side palisade-tissue is distinctly indicated. The single median bundle, which is evident projecting from the sclerenchyma into the adjacent soft tissue, retained this relative position throughout the bract. Usually, it has vanished along with the soft tissue. Its vessels were spiral and annular. The general form and extent of the parenchyma in the horizontal part of the bract is indicated in the diagram Text-Fig. 1. The thickness of the entire bract is there under-, rather than over-estimated.

The Sporangiophores. In an external view these organs must have appeared to be practically in the axils of the bracts, though as will be realized from an examination of the oblique nodal section, Fig. 7, they did not become completely free for a short distance above the insertion of the bract.

The sporangiophore itself was very similar to the typical form found in *Calamostachys*. The stalk was straight, and in a transverse section generally showing the form of a square with rounded angles, and with its diagonals placed radially and tangentially—a form obviously due to the pressure of the four sporangia which surrounded it. The structure of the stalk is very imperfectly preserved. Fig. 5 (*sp. tr.*) shows the median vascular bundle fairly well, while a few sections show very imperfect traces of it nearer the distal end. All efforts to determine its orientation have been unavailing. Surrounding the bundle is a regular mass of tissue, of more or less longitudinally elongated elements, bounded by a simple epidermis. In this tissue I have not been able to trace the phloem. A considerable portion of this ground-tissue consists of the elongated dense black elements which have been referred to above (p. 376). Usually, nearly all the cells between the bundle and the sub-epidermal layer on the outer side of the stalk are of this type, while there are commonly some on the inner side as well. They may be symmetrically disposed round the bundle, or even mainly on the inner side.

The peltate head is preserved only in fragments. The most nearly complete example is that of Fig. 4. The mushroom-like form is well shown, and the thick black external covering of long palisade-cells. The space below this was doubtless (from analogy) occupied by soft parenchyma in which the stalk bundle divided into four branches, one for each sporangium. There is evidence that the outline of the head was undulating, as indicated in Text-Fig. 1.

The Sporangia. As already indicated, each of the sporangiophores had four sporangia round it, so that there were normally seventy-two

sporangia in a whorl. The four belonging to one sporangiophore were set so that two were inner and two outer, or, as would perhaps be more accurate, two superior and two inferior. The whole of the sporangia were closely packed so as to fill completely the space enclosed between the bracts and the axis. Consequently as seen in radial longitudinal section they were roughly triangular, the outer ones base downwards, the inner base upwards (Text-Fig. 1). Seen in transverse section they appear as truncated wedges, the radial length of which will obviously vary with the position of the section in relation to the nodes (Text-Fig. 2).

The wall of the sporangium is exactly similar to that found in *Calamostachys*. It consists of a single layer of cells which are characterized by 'buttressed' walls, such as are familiar in the latter genus (Fig. 17). It is difficult to distinguish between buttresses and radial walls, and consequently to make out the real form and size of the cells. As far as can be made out these cells are brick-shaped, or lozenge-shaped in surface view, about 35 μ in the shorter and 100 μ in the longer diameter.

The thickening was practically confined to the inner and radial walls of the cells. I have seen no indications of a tapetal layer, but this could scarcely be expected in view of the state of preservation and ripeness of the cones.

Williamson,[1] in his description of the cone, makes a point of the partial or entire absence of the sporangial walls in some sections, and regards this as probably due to resorption as a means of liberating the spores in the absence of elaters. This seems to me quite an unnecessary assumption, in view of the fact that decomposition had proceeded to the extent of removing nearly all the soft tissue in the cone.

Attachment of the Sporangia. No details of this can be given, but the main fact is certain, that they were borne on the under surface of the peltate expansion of the sporangiophore, near its periphery.

The Spores. There is no indication of heterospory, no variation in the size of the spores in one or in different sections being noticeable. The spores are normally circular in form, with a diameter of about 80 μ. A characteristic feature, in the existing material, is that each spore shows two distinct walls, separated by an interval, besides which there is generally a large rounded or irregular dark mass in the centre.

The outer walls of adjacent spores are closely packed together, and consequently polygonal in section. The inner, which has a diameter about three-quarters that of the outer, is usually round. The most probable interpretation appears to me to be that the two walls merely represent the extine and intine, the latter having shrunk away from the former owing to a process of plasmolysis which would not improbably take place in the peaty mass of material before or during its calcification. In the same way

[1] Williamson, '87, p. 53.

I interpret the dark mass in the centre as the shrunken and carbonized remains of the cell-contents.

Williamson,[1] however, regarded the outer wall as belonging to the spore mother-cell, and stated that each mother-cell contained a single spore. This view would be possible if we regarded the spores as megaspores. Against this we have the fact that they would be rather small—they are just about the same size as the spores of *Calamostachys Binneyana*—and, further, in view of the fact that the 'mother-cell' wall is still not degenerated, we might fairly expect to find traces of the remaining spores of the tetrad, such as are found in *C. Casheana*. A careful search has failed to show anything which could possibly be interpreted in this way. I think it would also be rather surprising that all the cones should be macrosporous. Hence in the evidence available I see no ground for the assumption of heterospory, which this view seems to involve.

There are no other points regarding the spores which call for comment.

Affinities of the Cone. It is not intended to enter into any elaborate discussion of this question, since I do not consider that it can profitably be done without a complete reinvestigation of our knowledge of the fructifications of the Calamarieae. The only point which is really vital to this paper is whether or not the new features of the cone, now brought to light, necessitate such a revision of our ideas as to its affinities as would cause us to take it out of the genus it is supposed to belong. The Equisetales and Sphenophyllales, which groups I regard as more closely associated than generally suggested, all agree in having cone-like fructifications, in which the sporangia are borne on stalked sporangiophores; rarely directly on leaf-like organs. The cones of the two phyla broadly differ, those of the former having sporangiophores attached to the *axis* of the cone, while in the latter the sporangiophores are attached to the leaf-like bracts. Further, the Calamarieae have typically four, the Sphenophylleae one or two sporangia on each sporangiophore. Of course the former, too, are generally without the centripetal wood possessed by the latter. The fact that the two cones more recently described by Dr. Scott, *Cheirostrobus*[2] and *Sphenophyllum fertile*,[3] between them reduce the separation of Calamarian and Sphenophyllaceous cones to a matter of the type of primary wood, does not affect the present question. *Palaeostachya* has only one character in which it in any way even appears to approach the *Sphenophyllum* group: its axillary sporangiophores. This feature we have seen to be totally deceptive, and hence we may confidently assert that our cone is not to be placed on the Sphenophyllaceous side of the Calamarian series. Among the Calamarian cones themselves, two other types are known from petrified material—*Calamostachys* and *Bornia*—while *Cingularia* must be made the type of a fourth group still unknown among our petrified remains. Since

[1] Williamson, '87, p. 53. [2] Scott, '97. [3] Scott, '05.

the only feature of importance which distinguishes the *Bornia* cones from *Calamostachys* is the entire absence of bracts, and as this feature is not constant in *Bornia* itself, the relations of that genus need not be separately considered. *Cingularia* offers no ground for detailed comparison, as its internal anatomy is entirely unknown. Only *Calamostachys* remains. Here there seems to be a fundamental difference. Nodes surrounded by bracts alternate with nodes surrounded by sporangiophores. So we have said. But I think there is already sufficient evidence to throw serious doubt on that statement. Renault[1] describes the vascular supply of his *Calamodendrostachys Zeilleri* in these words:—'Dans l'espèce que nous avons figurée on compte quatorze coins ligneux, chacun muni d'une lacune, i (Figs. 5 et 6); de chaque côté se trouvent de petits faisceaux ligneux, muni ou non de lacunes, i', qui après s'être élevés dans l'entre-nœud, au-dessus des points d'insertion des sporangiophores, *sp.*, *redescendent* pour y pénétrer en restant à la partie supérieure de la portion horizontale de ces organes. Les petits faisceaux dont nous venons de parler sont au nombre de quatorze, et forment, dans une grande portion de l'entre-nœud, une couronne très grêle discontinue en dehors du circle des faisceaux principaux; ils séparent de ces derniers au-dessus du verticille de bractées stériles immédiatement placé au-dessous du verticille fertile que l'on considère, s'élèvent verticalement dans l'entre-nœud, puis redescendent pour pénétrer dans les pédicelles des bractées fertiles.' It seems scarcely possible to doubt that the 'petits faisceaux' are the strict homologues of our sporangiophore-traces. As in *Palaeostachya*, they are equal in number to the principal bundles, they separate from them 'au-dessus du verticille de bractées stériles,' they ascend vertically in the internode, and are then reflexed to pass into the sporangiophores. The reflexed portion of the trace is not carried so far down as in *Palaeostachya*, and the traces are not radially opposite the principal bundles, but alternate with them. These appear to me to be the only two structural differences of classificatory importance between *C. Zeilleri* and *Palaeostachya*.

In our English *Calamostachys* it was commonly assumed that the sporangiophores represent independent fertile whorls. The influence of the ventral-segment theory of the sporangiophore has thrown doubt on this. Williamson and Scott[2] noticed that the principal bundles passed the fertile 'nodes' without interruption of their protoxylem canals, and state that 'the fertile nodes . . . . show scarcely any modification of structure as compared with the internodes.' In a fine longitudinal section of a *Calamostachys* with the pith preserved (Manchester Museum Coll. Q. 295), it is a striking feature that the pith cells are 'shortened-up' at the level of the bracts, while they pass by the sporangiophores unchanged. These facts appear to me to raise the strongest doubt against the view that the

[1] Renault, '96, p. 130, Pl. LX, Figs. 3–8. [2] Williamson and Scott, '94, pp. 905–6.

sporangiophores represent independent whorls. I feel convinced that it will be found that the sporangiophore traces really arise just above the bract-traces, and then rise in contact with their parent-bundles till they turn out into the appendages to which they belong. If this view is correct, then the only important differences between *Calamostachys* and *Palaeostachya* are the absence of any considerable reflection of the sporangiophore-trace in the former, and the possession of (approximately) two bracts to each sporangiophore. I do not think that much stress should be laid on either of these differences. Among various Calamite cones the sporangiophore may be found attached to almost every part of the internode. The number of bracts in the whorl appears to have been anything from that of the sporangiophores up to three or more times as great as the latter.[1] It is noteworthy that although the specimens of *Calamostachys* with structure preserved have *approximately* two bracts to each sporangiophore, the relation is seldom exactly two to one, while in *Palaeostachya* it is possible that the number of bracts somewhat exceeded that of the sporangiophores. A tendency to multiply the number of appendages in each whorl seems to have been a characteristic Calamarian feature. Hence I would conclude that *Calamostachys* and *Palaeostachya* need not be remotely separated.

An interesting suggestion as to a possible explanation of the course of the sporangiophore trace in *Palaeostachya* was made to me by Mr. Boodle, to the effect that the triangle of sclerenchyma which is seen in longitudinal section between the ascending and descending limbs of the trace may be an upgrowth of the sclerized tissue of the disc, which has carried the trace with it. Against this view, however, I would urge the following considerations: Firstly, in *Calamodendrostachys* there is already a real, though small, reflection of the trace, while no sclerized tissue is present. Then the tissue referred to appears to me to be associated with the trace itself more intimately than with the disc. I have pointed out (p. 378) that in the sporangiophore-stalk there is a mass of dense resistent tissue which completely or partially surrounds the bundle, and that is a continuation of the strand of similar tissue which lies under the bundle in the 'disc.' No section exists good enough to show clearly the nature of the triangle of 'sclerenchyma' referred to above, but I strongly suspect that it consists, in part at least, of that same strand of tissue following the bundle and reflected with it. I think Fig. 21 accords with this view as well as can be expected. Finally, the only explanation of such an upgrowth would be a mechanical one, and I should doubt if any appreciable gain in rigidity is obtained in this way, though I would not profess to understand the remarkable mechanical adaptations of this cone. In view of all the facts at present available, the phylogenetic explanation seems to me much more probable, though even if the alternative view should prove correct, the

[1] Weiss, '76, Taf. xvi, Fig. 3 B, *Calamostachys germanica.*

close relationship of *Calamostachys* and *Palaeostachya* would not be seriously diminished.

Lignier[1] has already ably advocated the view that the 'fertile whorl' of *Calamostachys* is merely a displaced series of fertile segments of the sterile leaves, and has pointed out the significance of the course of the traces of the appendages in these fructifications. In the same paper he interprets the tetrasporangiate sporangiophore as the result of the fusion of adjacent sporangiophores in pairs, so that the compound organ now lies superposed to the interval between the pair of leaves to which its component sporangiophores belonged, while the total number of sporangiophores is reduced to half that of the bracts. This ingenious interpretation affords an admirable explanation of the structure of *Calamodendrostachys* and of some specimens of *Calamostachys*. In the numerous specimens of the latter genus in which the sporangiophores are more than half as numerous as the bracts, it will be more difficult to apply, while in *Palaeostachya vera* it cannot be applied. It does not appear to me that the relationship between the number of bracts and of sporangiophores in Calamitean cones is as definite as would appear to be commonly supposed. The only rule which seems to hold generally is that the sporangiophores are always equal in number to the primary bundles of the axis, or practically so, while the bracts are equally or more numerous. Thus, while agreeing with Lignier as to the probable origin of the sporangiophore and the mode of multiplication of the appendages, I think it must be allowed that the sporangiophores and bracts, having become separated, might be multiplied independently and sometimes irregularly. I should therefore regard it as more probable that the double number of bracts as compared with sporangiophores in *Calamostachys* is due to the bracts having undergone more division than the sporangiophores, rather than as due to a process of refusion among the latter.

Regarding the propriety of retaining our cone in the genus *Palaeostachya*, we may note that there is nothing in Weiss's[2] definition to exclude it: 'Stem and branches with asterophyllitiform leaves. Cones attached at the nodes in pairs (or singly ?), cylindrical, articulated. Bracts free, arcuate; sporangia attached to a straight columella or sporangiophore arising out of the axils of the bracts, elliptic, verrucose. Sporangiophore with peltoid apex, four inwardly-directed sporangia (secundum Renault).' In two respects the specimen now described does not fit in with the conception of *Palaeostachya* which up to now has been prevalent. The axillary position of the sporangiophore appears to be a secondary one, and consequently the cone does not provide a link between the Sphenophyll cones and *Calamostachys*, but represents a further modification of the latter type; while the number of bracts is certainly not double that of the sporangiophores. But

[1] Lignier, '03.

[2] Weiss, '76, p. 103.

neither of these features belongs to Weiss's original conception. The latter arose as an error of observation, the former was an assumption to satisfy a theory. Our own specimen and Weiss's type of *Palaeostachya elongata* seem to agree in all points of generic importance observable in both, and therefore I see no reason why they should not share the same genus until some vital distinction can be proved.

Solms Laubach [1] has placed Renault's *Volkmannia gracilis* in this genus, and it undoubtedly has (approximately) two bracts to each sporangiophore, but I do not think that reference makes that character an essential feature of the genus. Weiss's 'genera' can only be regarded as types, not as genera in the true sense. It will be rather surprising if all Calamitean cones with axillary sporangiophores are ultimately found to come within the limits of a single biological genus.

At least, so long as Weiss's classification is adopted there can be no doubt that this cone must be placed in his genus *Palaeostachya*, which he makes for cones with sporangia attached in fours to peltate sporangiophores which arise in or near the axils of the bracts. The course of the sporangiophore-traces may be added as a further generic character. Finally, it is of interest to note that Weiss himself suggests that both *Cingularia* and *Palaeostachya* arose from *Calamostachys*, by the movement of the sporangiophores upward in the former, downward in the latter case.

Summary.

Palaeostachya vera is a Calamarian fructification characterized by axillary sporangiophores.

Cones cylindrical, pedunculate. Diameter about 14 mm., length unknown.

Axis with external diameter of 4 mm., the vascular cylinder being about 2·7 mm. Primary vascular bundles generally eighteen, in pairs, probably not alternating at nodes, each with a protoxylem (carinal) canal on inner side, which is obliterated at the nodes. Secondary wood present at nodes only. Bract traces leaving bundles nearly at right angles, not divided. Sporangiophore-traces arising immediately above bract-traces, passing outwards very obliquely through the secondary wood of the parent-bundle, ascending in contact with bundle through half-internode, then redescending obliquely to enter the base of the sporangiophore.

Cortex mainly parenchymatous, with a sclerized ring above each node (the 'disc') perforated by nine unsclerized patches (the 'disc-canals').

Bracts approximately equal in number to the sporangiophores (generally eighteen), with a single bundle, sclerized ventral and parenchymatous dorsal portion. Lanceolate. Free. Sporangiophores peltate,

[1] Solms Laubach, '91, p. 332.

bearing four sporangia each. Sporangia flattened in transverse, triangular in vertical section. Walls consisting of a single layer of buttressed cells as in Calamostachys. Spores about ·08 mm., all of one kind.

In concluding this paper I desire to express the thanks I owe to Dr. D. H. Scott, F.R.S., for valuable suggestions, the loan of his own slides, and facilities for the examination of those in the Williamson Collection, and to Prof. F. E. Weiss, D.Sc., for the loan of the slides in the collection of the Manchester Museum, Owens College.

LITERATURE REFERRED TO.

LIGNIER, O. ('03): Equisétales et Sphénophyllales. Leur origine filicinéenne commune. Bulletin de la Soc. Linn. de Normandie, 5e sér., 7e vol. Caen, 1903.

RENAULT, B. ('96): Flore Fossile du Bassin Houiller et Permien d'Autun et d'Épinac, Pt. II. Paris, 1896.

SCOTT, D. H. ('97): On the Structure and Affinities of Fossil Plants from the Palaeozoic Rocks. I. On *Cheirostrobus*. Phil. Trans., vol. 189 B, 1897.

———— ('00): Studies in Fossil Botany. London, 1900.

———— ('05): On the Structures and Affinities of Fossil Plants from the Palaeozoic Rocks. V. On a New Type of Sphenophyllaceous Cone (*Sphenophyllum fertile*) from the Lower Coal Measures. Phil. Trans., vol. 198 B, 1905, pp. 17–39, Pls. III–V.

SEWARD, A. C. ('98): Fossil Plants, Pt. I. Cambridge, 1898.

SOLMS LAUBACH, GRAF ZU ('91): Fossil Botany. Oxford, 1891.

WEISS, C. E. ('76): Abhandl. der geol. Specialkarte von Preussen und den Thüringischen Staaten. Bd. ii, Heft 1, Berlin, 1876; Bd. v, Heft 2, Berlin, 1884.

WILLIAMSON, W. C. ('69): On a New Form of Calamitean Strobilus from the Lancashire Coal Measures. Mems. Lit. and Phil. Soc. Manchester, Ser. 3, vol. iv, 1871, pp. 248–65. Pls. VII–IX.

———— ('87): Organization of the Fossil Plants of the Coal Measures. XIV. The True Fructification of Calamites. Phil. Trans., vol. clxxix B, 1888, pp. 47–57, Pls. VIII–XI.

———— and SCOTT, D. H. ('94): Further Observations on the Organization of the Fossil Plants of the Coal Measures. I. Calamites, Calamostachys, and Sphenophyllum. Phil. Trans., vol. clxxxv B, 1894, pp. 863–959, Pls. LXXII–LXXXVI.

EXPLANATION OF PLATES XXXII AND XXXIII.

Illustrating Mr. Hickling's paper on *Palaeostachya vera*.

The letters attached to the catalogue-numbers of the slides are as follows: Q. = 'Cash' Collection (Manchester Museum); R. = Manchester Museum (general) Collection; S. = Scott Collection; W. = Williamson Collection (British Museum).

PLATE XXXII.

Fig. 1. Oblique transverse section. On left, in 'disc'; on right, below. *Br.* = bract, sclerized tissue of ventral surface; *Br'* = soft tissue of ventral portion of bract; *C* = portion of cortex, immediately below bract; *Ptxy.* = protoxylem mass obliterating canal at node; *2ny.xy.* = secondary wood: note how this decreases rapidly as section ascends. S. 474. × 16.

Fig. 2. Longitudinal (slightly oblique) section through node showing 'arching' of secondary tracheids over node. Section is not quite median through node. It shows the relation of 'disc' to node. *D.* = 'disc'; *n.* = node; *Pt.xy. C.* = protoxylem canal of bundle. W. 1573. × about 38.

Fig. 3. Transverse section of axis at node. Note large amount of secondary wood; absence of protoxylem canals. R. 143. × about 38.

Fig. 4. Peltate expansion of sporangiophore. R. 31. × about 38.

Fig. 5. Sporangiophore cut longitudinally, showing its bundle, *Sp. tr.*, in lower portion, where it adjoins cells of disc. R. 30. × about 38.

Fig. 6. Oblique transverse section of part of disc showing curved sporangiophore-trace crossing it. *Sp. tr.* = sporangiophore-trace; *M.* = tissue described at p. 376. Q. 336. × about 38.

Fig. 7. Oblique 'disc' section. At right edge, section cuts near lower limit of 'disc'; plane of section rises towards left edge. Note the ring of buttresses formed by the pairs of sporangiophore-traces and their accompanying sclerenchyma. *Br.* = base of bract; '*L. Can.*' = large 'disc canals'; '*Sm. Can.*' = small 'disc canal' (= torn-out sporangiophore-trace); *Sp.* = sporangiophore-stalks cut at various levels; *Sp. tr.* = sporangiophore-trace, descending limb; *Sub-ep. Scler.* = strand of sub-epidermal sclerenchyma. R. 30. × about 11.

Fig. 8. Oblique transverse section through lower portion of part of disc, showing large 'disc canal' half filled with parenchyma. *Par.* = parenchyma in 'disc canal'; *Phl.* = secondary phloem. R. 140. × about 38.

Fig. 9. Internodal transverse section. Above level reached by sporangiophore-trace. Shows small amount of wood in bundles as compared with that at nodes. Cf. Figs. 1 and 3. Q. 332. × about 38.

Fig. 10. Bases of two sporangiophores just emerging from disc. Part of section, Fig. 7. *M.* = tissue described at p. 376; *Pd.* = periderm; '*Sm. can.*' = 'small outer canal' (= torn-out sporangiophore-trace). R. 30. × about 38.

Fig. 11. Portion of internodal transverse section with cortex preserved. R. 142. × about 38.

Fig. 12. Portion of transverse 'disc' section. *Px. C.* = protoxylem canal of *main bundle*; *Sp. Fx. C.* = protoxylem canals of sporangiophore-traces (really more distinct than they appear in photo), see drawing, Fig. 16. Q. 336. × about 38.

Fig. 13. Similar section at slightly lower level. Each of the three protoxylem canals is seen to be elongated. They are dividing into the canals of the main bundles and those of the sporangiophore-traces. R. 34. × about 38.

PLATE XXXIII.

Fig. 14. Typical tracheids. *A*, annular, from sporangiophore-trace. Q. 337. × about 200. *B*, scalariform, from secondary nodal wood. S. 474. × about 280. *C*, pitted, from secondary nodal wood. R. 30. × about 280.

Fig. 15. Transverse section through vertical portion of bract. *Scl.* = dorsal sclerenchyma. R. 32. × 66.

Fig. 16. Uppermost bundle shown in Fig. 12. The protoxylem canals of the main bundle and the sporangiophore-trace are here clearly seen. Q. 336. × about 150.

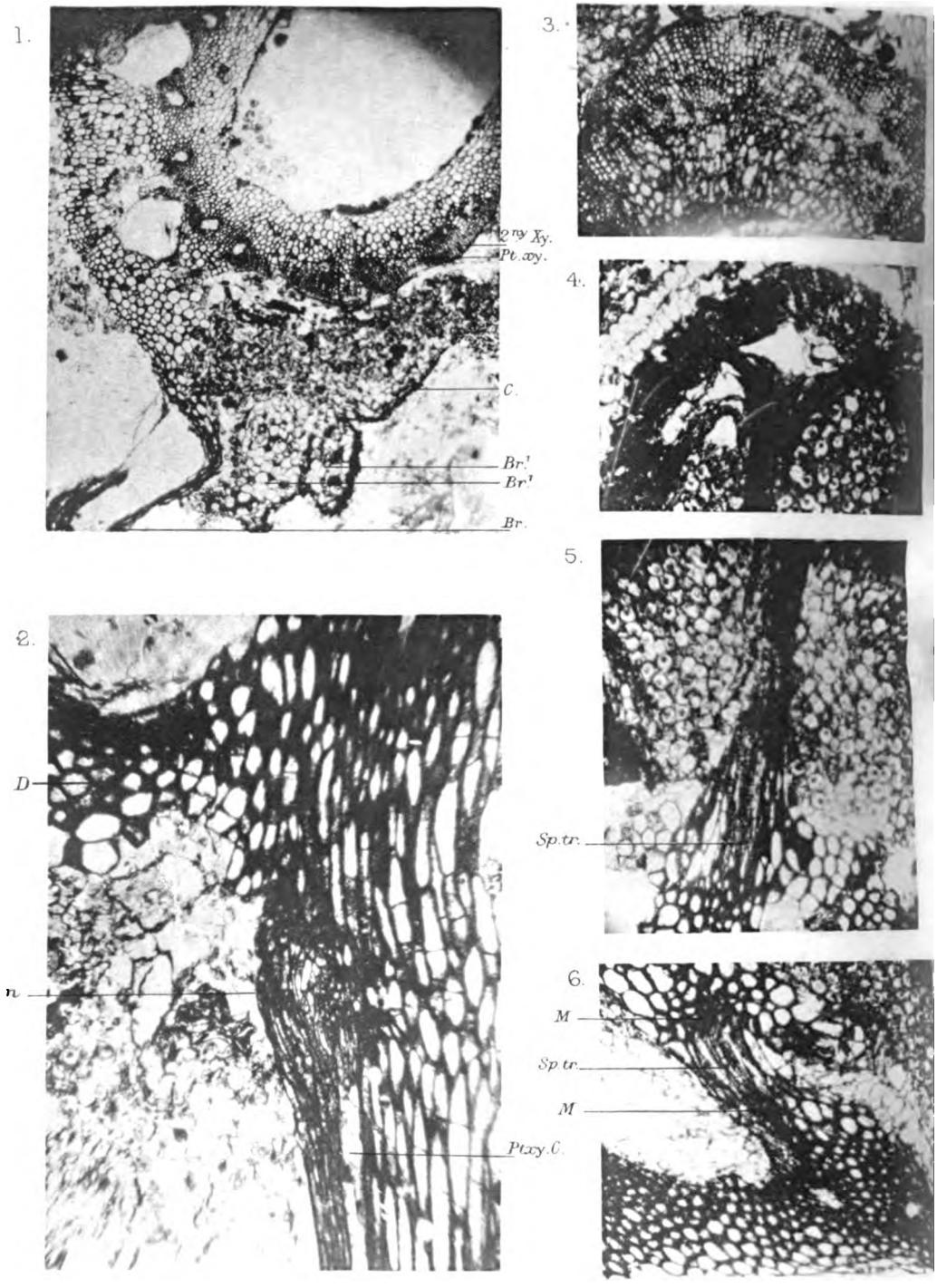
Fig. 17. Portion of sporangium wall in tangential section showing form of cells and 'buttresses.' R. 31. × 146.

Fig. 18. Tissue accompanying sporangiophore-trace, in transverse section through 'disc.' R. 144. × 120.

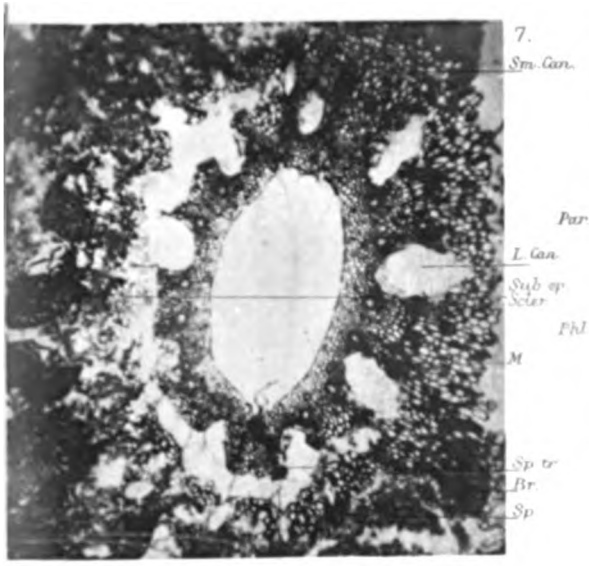
Fig. 19. The sporangiophore-trace bundle shown in Fig. 6. Q. 336. × about 136.

Fig. 20. Longitudinal section through node showing origin of bract-trace. *Pt.xy. C.* = protoxylem canal, obliterated at node; *D.* = part of 'disc.' W. 1573. × 70.

Fig. 21. Longitudinal section showing course of sporangiophore-trace and its accompanying tissue. Slightly oblique. *Pt.xy. C.* = protoxylem canal of main bundle; *S.Pt.xy. C.* = protoxylem canal of sporangiophore-trace; *Sp.* = sporangiophore; *Br.* = bract; *D.* = 'disc'; *D.C.* = 'disc-canal.' W. 1577. × 60.



HICKLING —— PALAEOSTACHYA.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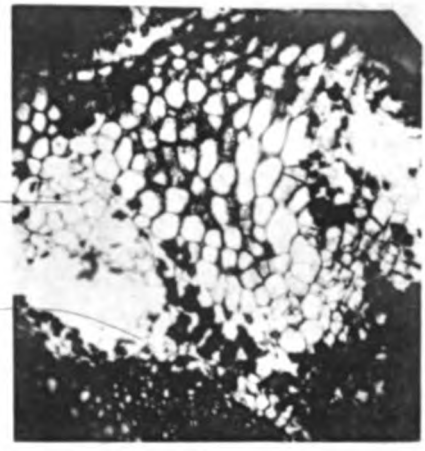
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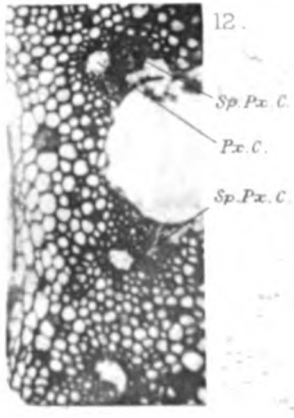
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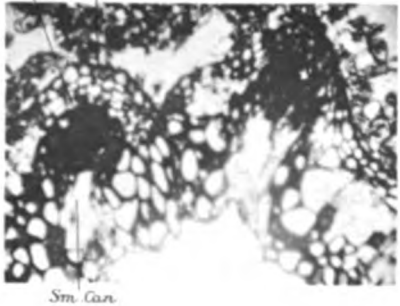
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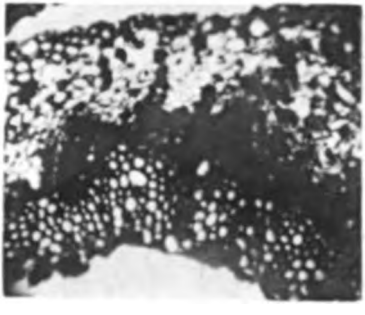
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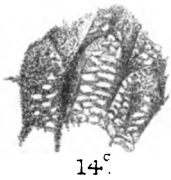

14c.

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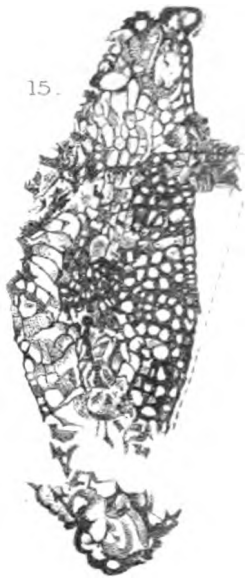

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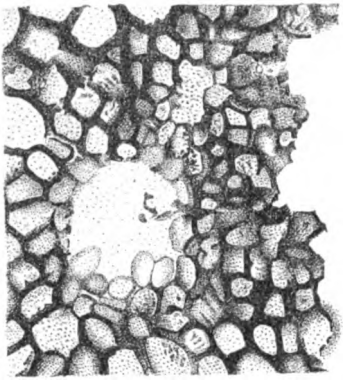

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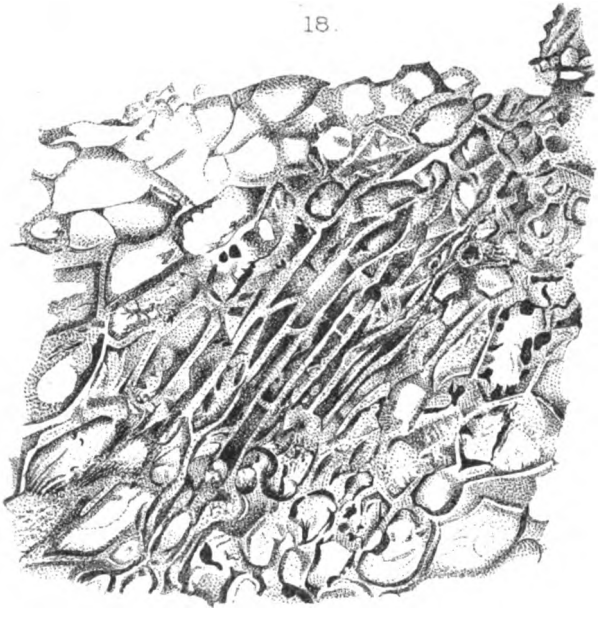

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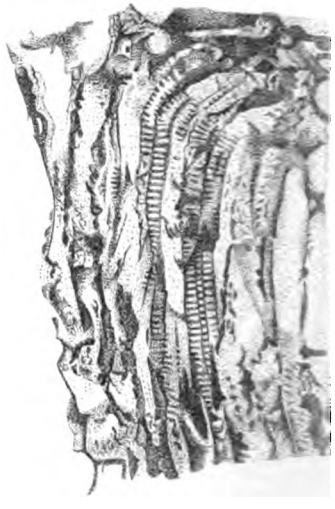

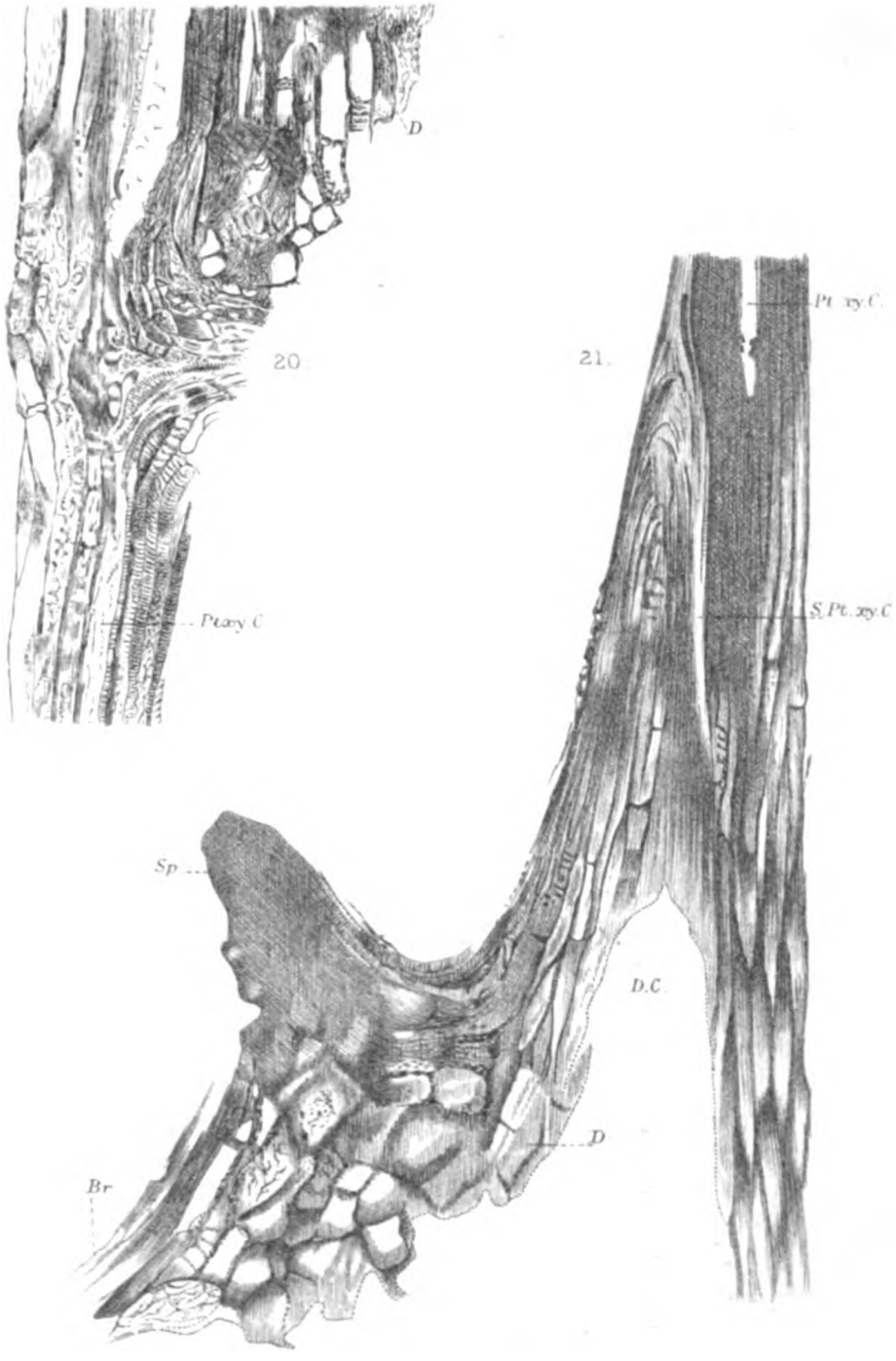
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On the Galvanotropism of Roots.[1]

BY

JESSIE S. BAYLISS, M.Sc. (BIRM.).

With Four Figures in the Text and two Curves.

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1. HISTORICAL.

ELFVING[2] observed that roots responded to electrical stimuli. He grew seedlings with the tips only of their radicles dipping vertically into water, between two electrodes, and found that the roots curved towards the positive electrode. He obtained the same result when the current traversed the root longitudinally and also with decapitated roots. With some seedlings (*Lepidium, Sinapis, Raphanus*) he could not get constant results, and with one (*Brassica*) the curve was always towards the negative

[1] An abstract of much of the work included in this paper was communicated to the Royal Society by Dr. Francis Darwin, Nov. 23, 1905.

[2] Elfving, Bot. Zeitung, 1882.

electrode. The currents used by him were so strong that the roots usually died.

In 1883, Müller-Hettlingen [1] published the results of his researches in the same subject. He used a horizontal glass plate, covered with a piece of flannel which dipped at opposite ends into water: on this flannel the seedlings were laid, and then covered with another glass plate which was prevented from touching the lower by glass supports: the current passed through the flannel, and so presumably through the roots. Here the curvature was towards the negative electrode, and these radicles seldom died.

Little reliance can, however, be placed on these experiments, for it is very uncertain how much, if any, current passed through the roots: also rheotropic, hydrotropic, and geotropic reactions were not rendered impossible.

Müller-Hettlingen's work was closely followed by that of Brunchhorst,[2] whose investigations seemed to reconcile these conflicting results. He drew the conclusion that the positive curvature observed by Elfving was due to the action of a strong current, while Müller-Hettlingen's negative curvature was due to the latter using a much weaker current. The positive curvature he considered to be of a traumatropic nature, due to injury of the anodal side of the root, whereas the curvature to the negative electrode he supposed to be the result of a directive influence, and to be analogous to geotropic and heliotropic curves.

In experiments with strong currents, Brunchhorst used a similar apparatus to Elfving, but when working with weak ones he used a glass cylinder rotating horizontally on a clinostat, thereby eliminating geotropic action.

The strength of the currents used by these investigators is not very definitely stated; Elfving used 2, 4, or 6 Leclanché cells, and Müller-Hettlingen 4 Grove's cells; but as these cells are liable to rapid and pronounced polarization when in constant use, the currents obtained could not have been of a very reliable or uniform nature.

Brunchhorst estimates the strength of the currents he used by the amount of deposit of copper per hour; but even this does not convey a very satisfactory idea of the current used. The current passed through water in which the roots were immersed, and since these and water have dissimilar resistances, the amount of current passing through them could not be determined from external measures of current strength. The current density in each root would depend as much upon the area of cross section of the surrounding water as upon the current strength in the external circuit.

[1] Müller-Hettlingen, Pflüger's Archiv, xxxi, 1883.
[2] Brunchhorst, Ber. d. Deutsch. Bot. Gesellsch., 1884.

Further research of a less restricted character was obviously needed [1], and this investigation was initiated and carried on in the Botanical Laboratory of the University of Birmingham, under the direction of Dr. (now Professor) A. J. Ewart, to whom the author is greatly indebted for much kind and valuable assistance. She also wishes to express her thanks to Professor Hillhouse for the use of the resources of the Botanical Laboratory and for his kindness in criticizing the work, and also to Professor Poynting for the use of apparatus belonging to the Physical Department and for his help when referred to on points of technical detail.

2. Preparation of Material.

To prepare material for the following experiments seeds of *Vicia Faba* (both broad bean and horse bean), *Phaseolus multiflorus*, *Zea Mais*, *Pisum Sativum*, *Helianthus annuus*, *Helianthus giganteus*, *Cucurbita Pepo*, and *Ricinus zanzibarensis* were soaked in water for usually twenty-four hours.

The seeds after soaking were germinated for a further twenty-four hours in moist sawdust, then carefully withdrawn, and the germination continued for a time in a vertical position in a damp chamber so as to avoid the possibility of accidental or injury curvatures. As an alternative their germination was continued horizontally in a moist chamber which was rotated on a clinostat.[2] To ascertain whether the radicles grew, or not, after stimulation, the apical centimeter was marked with Indian ink into spaces 1 mm. wide.

3. Experiments with Seedlings growing in Gelatine.

Instead of using water, as in Elfving's and Brunchhorst's experiments, seedlings were arranged in a glass vessel containing 3 per cent. gelatine between two platinum electrodes, 10 to 12 cm. apart, and a current of 4·2 voltage passed through. In order to trace the limits of acid and

[1] See Pfeffer, Physiology of Plants, vol. iii, Eng. trans., section 42.

[2] The clock clinostats used in these experiments are a hitherto undescribed adaptation, by Professor Hillhouse, of American eight-day clocks, in which the full eight-day working power is retained—a point of some importance in prolonged experiments, since disturbance for winding purposes is reduced to a minimum. The face, hour-hand, and spindle are removed, and a new toothed wheel on the minute spindle engages another wheel borne upon a new spindle, of the same thickness as the minute spindle, which is fixed upon the clock framework a short distance away. The rate of rotation of this second spindle depends upon the relative number of teeth in the two wheels. A perforated brass disc, or table, fits upon either of these spindles by means of a split sleeve, and thus each clock gives rotation at one-hour periods, and at longer or shorter periods according to construction. For safety, the face is replaced by a metal plate, pierced for spindles and key. To exclude water when in horizontal use, another plate, with slit for the spindle, can be slipped on. With vertical axis, this clinostat carries freely upon its rotating disc a weight of several kilos. Its use with horizontal axis is illustrated, in an extreme case, in the accompanying Fig. 2, a flat cork of suitable size being then attached to the disc by means of the perforations. In a more recent form of the instrument, each clock bears two supplementary spindles with different rates of rotation.

alkaline diffusion from the respective poles the faintly alkaline gelatine was tinged slightly with phenolphthalein. After about twenty hours the roots in the strongly alkaline and acid zones near the negative and positive electrodes respectively, showed curvatures towards those electrodes, and after a further twenty hours the roots in the intermediate regions curved also but to a lesser degree, and then chiefly to the negative electrode.

Thus, in one experiment, with 15 seedlings after 48 hours, 9 curved to the alkaline pole, 2 to the acid, and 4 remained straight, while with another set of 11 seedlings, 5 curved to the alkaline pole, 4 to the acid, and 2 did not curve. These radicles were situated at distances varying from

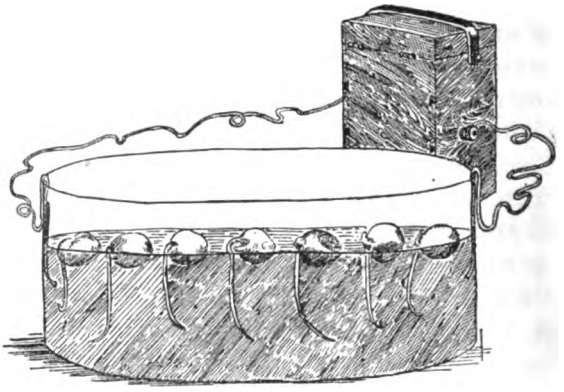


FIG. 1. Seedlings growing in gelatine.

·5 to 12 cms. from the electrodes (see Fig. 1). For some experiments the gelatine was rendered slightly acid by a few drops of H_2SO_4. The greater number of curvatures were still to the alkaline pole, even in the intermediate region, which was now slightly acid instead of alkaline.

To ascertain how far geotropic irritability was affected by growth in gelatine, a few seedlings were laid horizontally in that medium, and compared with a control set grown in a damp, well-aerated vessel. After seventeen hours the control seedlings had curved nearly 90°, while those in gelatine were still straight ; and only after two days did the latter begin to curve slightly, no doubt because their geotropic sensitiveness was in large measure suppressed by lack of proper aeration.

Some of these experiments were now repeated, using 4 per cent. gelatine with holes cut in and kept open by means of small chips of wood. Here the better aeration brought about a quicker response, for the roots in the zone intermediate between the strongly acid and alkaline zones showed decided curves within the first twenty-four hours after the current had been put on.

Through one set of radicles growing in gelatine a very strong electric current (220 volts) was passed for a few minutes, and then the radicles were wiped and divided longitudinally at right angles to the direction of the current and pressed on to litmus-paper, but no trace of any internal differential polarization could be detected. Both halves of those radicles taken out of the strongly alkaline region contained local acid areas surrounded by alkaline ones: in the strongly acid region both halves were strongly acid, and an acid reaction similar to that of unstimulated roots was all that could be detected in the intermediately placed roots.

4. Experiments with Polarizable Electrodes directly applied to the Root.

But more definite results were found by placing the electrodes directly on the roots.

Strong currents were applied by means of tapering platinum electrodes touching appropriate points on the root, which was kept moist during the process. The voltage used in such cases was 4·2, but the current naturally depended mainly upon the variable resistance of the interposed tissues.

After marking with Indian ink the side (not the place) which the

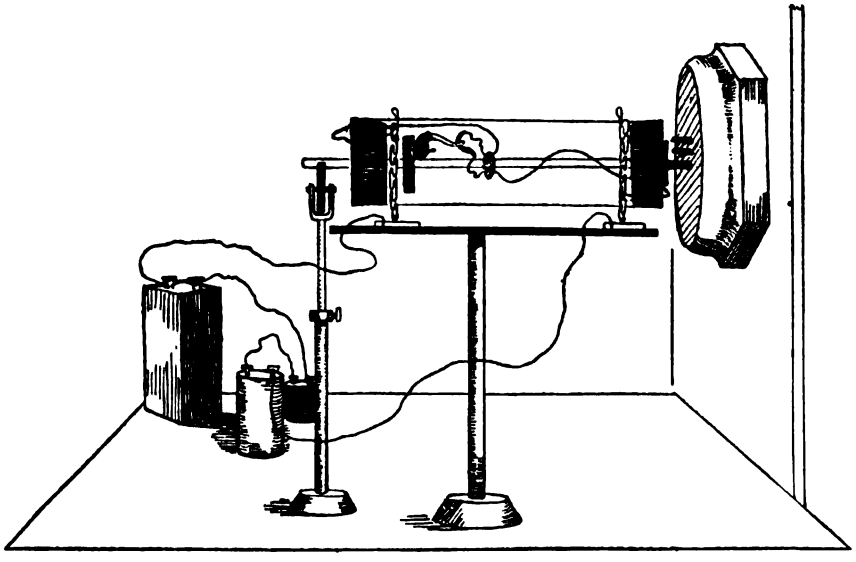


Fig. 2. Diagram showing apparatus used when passing a weak constant current through a root.

positive electrode had touched, the seedlings were pinned to a cork (perforated for aeration) which fitted into a small moist chamber lined with moist blotting-paper. This chamber was now attached to a clinostat and

so placed that the roots were horizontal and parallel to the axis of rotation, and at right angles with the incident light.

When working with very *weak currents* a more complicated apparatus was used. A glass cylinder 30 cm. × 8 cm. was fitted at either end with a cork, through which a glass rod was passed. This cylinder was so attached to the clinostat that the glass rod constituted a horizontal axis of rotation, and its end remote from the clinostat rested upon friction wheels (Fig. 2).

Surrounding the cylinder at each end was a ring of wire with radiating projections. Beneath each ring was a small vessel of mercury, and as the cylinder revolved the projections dipped into the mercury. Inside the cylinder the glass rod passed through a large cake of paraffin wax, which served as support for two small platinum electrodes, and wires from these passed out of the cylinder at opposite ends, and were joined up with those forming the rings. A cork disc arranged parallel with the other corks served to support the seedling, so that, when the electrodes touched the radicle, and the ends of the conducting wires from the battery were dipping in the vessels of mercury, a complete circuit was formed.

The same precautions as before were taken to ensure moist and oxygenated air.

For some time there was a great difficulty in getting a current which after passing for some hours did not kill the roots. For the supply of electricity an accumulator of voltage 4·2 was used, and in the circuit at first was placed a resistance box of 14,000 ohms. This current proved too strong, so water resistances, in the form of 1, then 2, then 3 metre-length tubes filled with very slightly acidulated water, were added. The current now seemed about the right strength, but was so feeble that it could not be measured even by a milli-ampere metre.

After ten days or so the water resistances did not act well, owing to polarization, and had to be discarded. Ultimately instead of the accumulator a dry cell, voltage 1·35, was used, and this with two resistance coils of 100,000 ohms. and 50,000 ohms. respectively, gave a workable current, and one easily measured from the formula $C = \frac{E}{R}$. The 150,000 ohms. resistance was so great as to render the other resistances negligible.

With these two kinds of apparatus electric currents were passed through several hundreds of seedlings—a tedious process, since from the nature of the apparatus only one could be treated at a time.

4 *a*. Results with Weak Currents.

Weak constant currents varying in duration from five hours to two days, when stronger than $\frac{4\cdot2}{150,000}$ ampere, gradually killed roots, but from

$\frac{4{\cdot}2}{150{,}000}$ to $\frac{1{\cdot}35}{150{,}000}$ ampere they produced a curvature to one or other electrode according to its position.

Of 13 seedlings, in which the positive and negative electrodes were placed exactly opposite one another, at about 1·5 mm. to 2 mm. from the root apex, 8 curved to the positive electrode, 4 curved to the negative electrode, and 1 between the two: there was frequently blackening and signs of injury, more especially at the place where the positive electrode touched.

To add more resistance and thus still further weaken the current, the electrodes were placed 1·5 cm. or more apart; and now the curvature was towards the apical electrode, whether positive or negative, and usually there was no sign of injury. The results from 15 seedlings tested in this way, during 15 nearly consecutive days, gave 13 curvatures to the apical electrode, whether positive or negative. Two only died; the others showed no sign of injury.

4 *b*. Results with Strong Currents.

With strong currents of 3 to 8 minutes duration voltage 4·2 and resistance that of the root tissue only, the results were as follows:—

α. With electrodes on opposite sides, at a distance from the apex varying from 1·5 to 5 mm., out of 29 radicles, 27 curved to the positive electrode, 1 remained straight, and the other curved to the negative electrode.

β. With electrodes on opposite sides, one placed 1·5 mm. from the apex and the other 5 to 10 mm. away, out of 21 radicles, 12 curved to the apical electrode, whether positive or negative, 4 remained straight, 3 curved in the opposite direction, and 2 were doubtful.

γ. With electrodes on the same side of the root, one 1·5 or 2 mm. from the apex, and the other varying from 5 mm. to 4 cm. away, out of 69 seedlings, 37 curved to the apical electrode, 14 curved away, 20 did not curve, and the other three died.

δ. With one electrode flat against the apex, and the other 1·5 to 3 mm. away, out of 33 seedlings, 16 curved to the current side (7 only slightly), 16 did not curve, 1 curved away.

ε. With one electrode flat against the apex, and the other more than 5 mm. away, out of 9 radicles none curved.

It was quite possible to get these positive and negative curvatures without visible injury to the root, even when examined microscopically, although in some cases the epidermis and perhaps one or two cortical layers of cells were injured, and in a few the injury extended even to the stele.

4*c*. Summary of Results with Polarizable Electrodes.

From these results it will be seen that a strong electric current applied to the root for a short time will produce just the same kind of curvature as a weak constant current for a long period, and that the direction of the curvature evidently depends on the position of the electrodes, not on whether the current is strong or weak.

If both the electrodes are on the sensitive zone of the root, and the same distance from the apex on opposite sides, the curve is always to the positive electrode.

If one electrode is on the sensitive zone, and the other some distance away, either on the same side or opposite side, the curvature is always towards the apical electrode, whether positive or negative. A few experiments performed without the use of a clinostat showed that electrical stimulation could produce curvatures even in opposition to the stimulus of gravity.

Some lateral roots were tested and gave similar results to primary ones.

4*d*. The Angle of Curvature.

The angles of curvature were usually sharp, and varied in extent from a few degrees to 180° or more, according to the duration and amount of stimulation (cp. Fig. 1). After about twenty-four hours or less the effect of the stimulus passed off; the autotropic tendency of the root would then assert itself, and the last centimetre or so would take an 'S' form. Sometimes when the stimulation had been feeble and the curve only slight the root straightened itself completely.

4*e*. The Sensitive Zone.

To find the length of the sensitive zone of the root the position of one or both electrodes was varied from time to time, and beyond the fourth or fifth millimetre from the apex no response to stimulation took place. The most sensitive part was at from 1 mm. to 2 mm., but a slight curve could be obtained even when one or both electrodes were placed 4 or 5 mm. from the root-tip.

4*f*. Decapitated Roots.

With decapitated radicles there was usually no response, either when decapitation took place before stimulation or when stimulation preceded decapitation, as the following results show:—

11 radicles with 1·5 or 2 mm. of their tips cut off were stimulated, and of these 8 remained straight, 2 curved slightly, and 1 curved 30°.

6 radicles were stimulated, and their tips (1·5 mm.) then cut off, and of these 5 did not curve.

In these experiments electrodes were applied from 1·5 to 2 mm. from the apex, and then just the tip or 1·5 mm. of the root was removed.

4*g*. Duration of Stimulus.

To find the duration of stimulus, roots were stimulated with strong currents, as in 4 *b* (*a*), fixed in plaster of paris, and rotated on a clinostat. On opening the casts after one or two days and again rotating, there was a curvature in an hour or so, but after three or four days in the plaster frequently no curvature took place. For example, 7 radicles were stimulated and put in plaster of paris, and released at the end of one or two days and all curved: whereas of 12 radicles similarly treated but released after three or four days, 7 remained straight and only 5 curved.

5. Experiments with Non-Polarizable Electrodes directly applied to the Root.

Some experiments were made, using non-polarizable electrodes with the platinum electrodes in the circuit, and at the same time control experiments, using platinum electrodes with the non-polarizable ones in the circuit, so that the total resistance was the same in both cases.

The non-polarizable electrodes were made by fitting a small brush of camel's hair into a tiny tube 1·5 cm. long, down the centre of which extended a copper wire from the accumulator: the brush was kept moist by diluted slightly acid cell-sap extracted from pressed roots, slowly flowing down from the glass tube. The current from the lighting main (220 volts) was used, and a large resistance of 2,200 ohms. in the form of three resistance lamps arranged in series was added.

The current was passed for fifty seconds; 54 roots were separately tested, and out of these 43 remained straight and 11 curved, but only slightly in most cases.

In the control experiment 40 roots were tested, and of these 9 remained straight and 31 curved, and these very frequently showed scars and other signs of injury.

The non-polarizable electrodes seldom caused any injury. When the 220 volt current was passed for longer than fifty seconds, even with the non-polarizable electrodes, a curvature took place.

6. Action of Local Acid and Alkali.

To ascertain whether there was any appreciable production of acid or alkali the cell-sap near the electrodes was tested with litmus-paper: there was a well-marked acid reaction at the positive electrode contact,

but at the negative electrode only a weak alkaline reaction was evident, this being probably due to partial neutralization by the CO_2 of respiration. These reactions suggested that possibly touching the sensitive zone of the root with acid or alkali might produce similar curvatures to those produced by an electric current, so pieces of blotting-paper, 1 sq. mm. in extent, soaked in either a solution of sodium carbonate or sulphuric acid, were placed on roots about 1·5 mm. from the apex, with the following results:—

Of 11 roots on which decinormal solution of the alkali was placed 8 curved towards it.

Of 42 roots treated with decinormal or weaker H_2SO_4, 31 curved to the acid, 6 away, and 5 did not curve.

Of 11 roots on each of which a tiny slice of electrolysed acid tissue had been placed 8 curved to the acid.

Of 11 roots on each of which a slice of electrolysed alkaline tissue (or the sap only) had been placed 8 curved to the alkali.

A few experiments were tried in which decinormal acid and decinormal alkali were placed opposite one another, when the curve was to the acid side (thus producing a curve analogous to the one obtained when the positive and negative electrodes were placed opposite one another).

These acid and alkaline curvatures could be obtained without *injury* to the root, although when the acid or alkali was strong injury *did* take place.

7. Latent Period of Response.

The response to the electrical stimulus is slow, and is not apparent to the naked eye until after at least three or four hours at a temperature of 19° to 22° C., while at a lower temperature (15° to 18°) often twelve hours or more elapse before there is any sign of a curve. With a horizontal microscope, however, a curvature can be detected within an hour of stimulation.

8. The Abnormal Thickening of many Roots.

Accompanying the curvature of the roots there was very frequently a thickening just above the electrode which was placed between the apex and the fourth and fifth millimetre space (Fig. 3). Microscopic sections of this showed merely a slight increase in the size of the cortical cells. This thickening appeared even when growth had been stopped for a few days by imbedding in plaster of paris.

This could hardly be a correlative action due to arrest of growth at the apex causing an increased tendency to lateral swelling behind, for when unstimulated root-tips were fixed in glass caps or glass collars no such result was produced. Hence this phenomenon must be due to the stimulatory action of the electric current, or more probably of the products

of electrolysis in the cell-sap, since a similar thickening frequently appeared in the neighbourhood of the stimulated spot when radicles curved as the

FIG. 3. Roots showing an abnormal thickening.

result of the external application of acid or alkali. The same kind of thickening took place when the tips of broad beans were allowed to grow against a pellet of blotting-paper saturated with a very dilute solution of sodium carbonate (Fig. 4).

Nemec[1] in a recent paper drew attention to a thickening of roots in the strongest growing part produced by allowing them to grow for an hour in a solution of either chloral hydrate or benzine, and then transferring to pure water: after forty-eight hours or so the normal growth was resumed again.

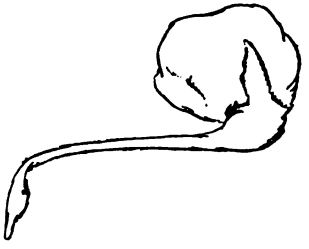


FIG. 4. A root of Broad Bean, showing an abnormal thickening due to growing against a pellet of blotting-paper saturated with dilute sodium carbonate. The curve is geotropic.

9. ABNORMALLY ELONGATED CELLS.

There should be mentioned another feature which was to be seen when any injury extending two or more cells below the epidermis occurred.

To the naked eye nothing was apparent except a brown scar, but microscopic examination revealed cells, two or three rows deep, with greatly extended radial walls; and between these cells and those forming the endodermis the cells were of the normal size, though the protoplasm in them appeared to have contracted and was probably dead. A few of these cells when measured had radial walls of lengths ·51 mm., ·68 mm., ·85 mm., and 1·53 mm., compared with the normal diameter of ·34 mm. or ·51 mm.: the tangential walls were nearly normal, and did not differ much from one another.

Injured places on roots which had been made to curve by the external

[1] Nemec, Pringsheim's Jahrbücher, vol. 39, p. 689.

application of acid or alkali, when examined microscopically, also showed a tendency in the cell walls to elongate radially.[1]

10. Rate of Growth of Stimulated Roots.

From time to time measurements were taken to see whether the normal rate of growth was disturbed by stimulation. The average rate of growth per hour during the twenty-four hours preceding stimulation was taken, and also during the twenty-four hours following it and during the next day: the results showed stimulation was followed by retardation.

The following measurements illustrate this:—

	Rate per hour during 24 hours before stimulation.	*Rate per hour 24 hours after stimulation.*	*Rate per hour 24 hours later still.*
Phaseolus multiflorus	·44 mm.	·24 mm.	·42 mm.
Vicia Faba	·14 „	·06 „	·23 „
Vicia Faba	·3 „	·25 „	·04 „
Phaseolus multiflorus	·66 „	·49 „	·17 „

In many cases where no curvature took place after stimulation there was a complete stoppage of growth. That growth took place on both sides of the root was readily seen by covering the root after stimulation with a thick layer of Indian ink, and observing the cracks which formed on the concave as well as on the convex side of the curved root.

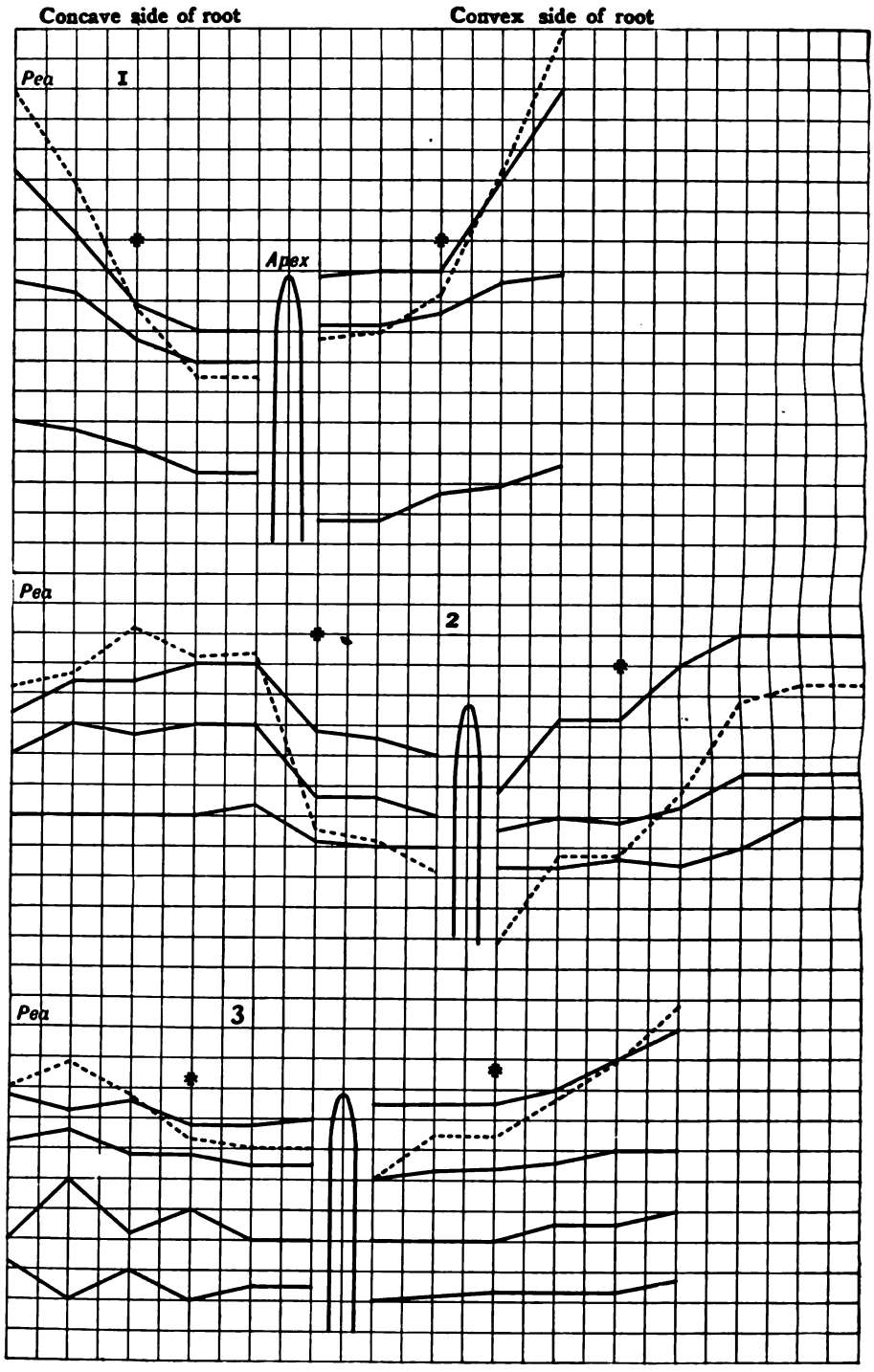
By using a horizontal measuring microscope, and taking periodical observations, the rates of growth on both sides of the curving root were more accurately measured. The readings were taken during the two or three hours preceding stimulation, and then continued for three or four hours.

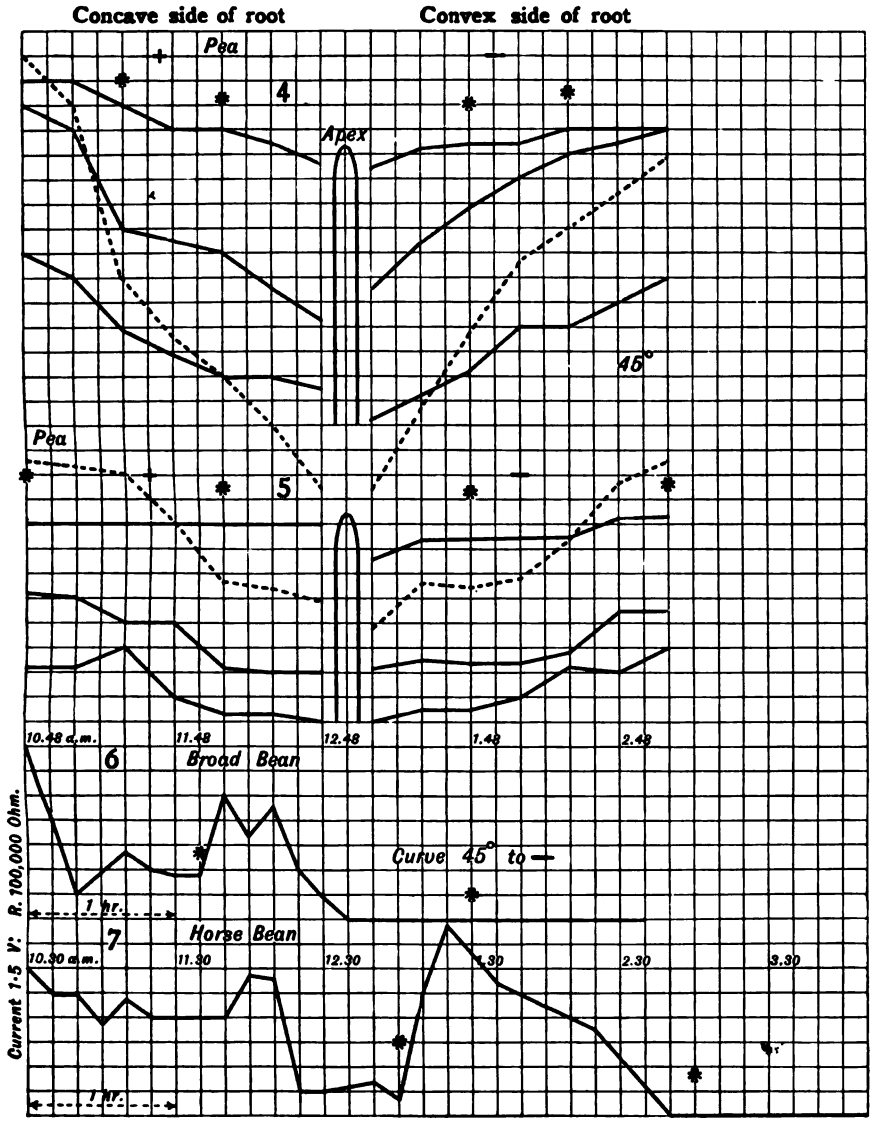
During the first and second hour or longer after stimulation, when prolonged weak currents were used, there was usually an acceleration of growth on both sides of the curve; but it was greater, and lasted longer, on the convex side. Ultimately growth was retarded, if not quite checked, on both sides of the root.

10*a*. Diagrammatic Representation of the Growth in Length of Curving Roots.

The following diagrams are selected from many to give an illustration of the curves of growth usually obtained. The three black lines to each figure except Figs. 6 and 7 represent the curves of the three apical zones marked out on each root, each zone of about 1·5 mm. length: the red line represents a general curve made by regarding the three zones as one. Each cm. length on the base line represents a period of one hour, and a cm. length on the ordinate line a unit of increment in length. The asterisks

[1] The question of these abnormal thickenings is being made a subject for further study.


Concave side of root
Convex side of root
Pea
I
Apex
Pea
2
Pea
3



NOTE.—The 'red' line mentioned on p. 398 is represented in this diagram by a dotted line.

mark the time at which a strong current (4·5 volts) was passed for three minutes; or, in curves where two appear, the period during which a weak current (1·5 volts R 100,000 ohms.) was passing.

The signs + and − indicate the sides of the root touched by the + or − electrode.

In each case the lines on the left-hand side indicate the concave side of the curve, and on the right the convex.

In Figs. 6 and 7 the curves represent the growth in length of the root as indicated merely by change in the position of the root-tip.

11. Experiments with Acid and Alkali diffusing through Gelatine.

Lilienfeld[1] has recently published some experiments of tropic responses induced by different salt solutions in radicles growing in gelatine. No very definite results were obtained, and in some cases they were even contradictory; yet a few might well be classed as of an ordinary chemotropic nature.

With a view to seeing whether there was any analogy between these curvatures, especially those produced by acid and alkaline solutions, and the curvature due to an electric current, similar experiments in a slightly modified form were made.

Seedlings were grown in a round glass vessel about 12 cms. wide containing a neutral solution of agar (5 or 6 per cent.) from the centre of which a small rod (·5 cm. diameter) of the jelly had been cut out, and the hole thus made was plugged with a roll of blotting-paper which was moistened with the solution to be tested, either H_2SO_4, NaHO, or sodium carbonate of $\frac{N}{10}$ or other strength, or phosphoric acid neutralized by KHO. In some instances the solid jelly was stirred up to ensure better aeration. Each experiment lasted about two days, during which time the blotting-paper was moistened several times. The atmosphere above the seedlings was kept damp and well aerated. A few of these experiments were carried out on a clinostat.

No definite results were obtained, probably because the method was incapable of proper control, diffusion taking place too rapidly to allow of one side of the root perceiving and responding to the stimulus before its whole surface was equally affected. An attempt to secure slower diffusion was made by plugging the stem of a very small thistle funnel with blotting-paper, and inserting it in the gelatine instead of the usual roll, but with no satisfactory results.

[1] Lilienfeld, Ber. d. Deutsch. Bot. Gesellsch., March, 1905.

Similar experiments were carried out using troughs of sand, silver or coarse red, the salt solution being placed at one end of the trough in a little cubical chamber, of which the side facing the seedlings was made of plaster of paris.

In these experiments also the results were very conflicting. Some rather better results were obtained when using two plugged thistle funnels containing, respectively, decinormal solutions of NaHO and H_2SO_4, and placed 10 to 12 cms. apart, with the seedlings in rows between them; the gelatine being coloured with phenolphthalein so that the extent of diffusion might be traced.

On the whole this series of experiments gave no conclusive results. Possibly the lack of aeration in gelatine delayed the curvatures until diffusion had gone too far; while in the troughs of sand the drainage and other currents may have been too rapid to allow of any difference of concentration being maintained on opposite sides.

The direct application of acid and alkali, however, on opposite sides gave very decided and consistent results.[1]

12. Summary of Results.

From the details which have been given there is enough evidence to connect these galvanotropic curvatures with those of a chemotropic character—the chemical stimuli being here the acid and alkaline ions formed during electrolysis.

In support of this view the following facts may be summarized:—

1. Acids and alkalies are formed in appreciable quantities at the places where the + and − electrodes touch the roots. (Section 6.)

2. Similar curvatures to galvanotropic ones are produced by acids and alkalies placed on the sensitive zone of the root. (Section 6.)

3. If a piece of root tissue under a + or − electrode be cut out and applied to another root, the latter curves to the acid or alkaline tissue. (Section 6.)

4. Acids and alkalies applied to the sensitive zone of roots can produce signs of injury similar to those produced by an electric current. (Sections 8 and 9.)

5. When one electrode is placed flat against the apex, and the other at some distance beyond the elongating zone of the root, there is no curvature: in this case the acid or alkali is produced in the root cap, and by diffusion affects the sensitive zone equally in all directions, and hence there is no differential response. But if the hinder electrode is on the sensitive zone, usually the root curves to this electrode, but sometimes remains straight,

[1] In the research as carried out, the experiments with seedlings grown in gelatine (section 3) were consequent upon this series of observations.

possibly because of the nearness of the electrodes to one another. (Section 4 *b*, *d* and *e*.)

6. When a current is passed through gelatine in which a number of roots are growing, both kinds of curvatures take place. (Section 3.)

7. When non-polarizable electrodes are used, with a strength of current sufficient to produce a curvature if applied by polarizable electrodes, no curvature takes place. (Section 5.) With these electrodes any acid or alkali would be chiefly formed in the glass tubes, and diffusion would hardly take place with sufficient rapidity for the acid or alkali to be long enough in contact with the root to cause a curvature.

13. Theory and Critical.

During the passage of an electric current through a root there are two influences to be taken into account :—

(1) The accumulations of ions near the electrodes, (2) the ions en route between the electrodes, either or both of which may be concerned in the tropic response.

Taking into consideration the action of either the acid or alkaline ions, it will readily be seen that a stimulus given by the accumulation, say, of acid ions will act in a direction quite contrary to that of travelling acid ions ; and thus in the case of a current across a single root between directly applied electrodes there would be four possible stimulatory actions coming into play, namely those due to the accumulated acid ions and the travelling alkaline ones acting in one direction, and the accumulated alkaline ions and the travelling acid ions acting in the opposite direction ; and the response would depend on whether one set of stimuli predominated over the other.

Since with transverse currents directly applied a curvature to the positive electrode is always carried out in preference to one to the — electrode, it is evident that the acid ions have a greater stimulatory effect than alkaline ones ; and this is what might be expected, since it is a well-known fact that acid ions travel, and hence accumulate, more quickly than alkaline ones, and also are more chemically active.

Where the current traverses the root longitudinally, the curvature no doubt is due to the stimulating action of whichever ions have accumulated in the sensitive zone, since this region, being the actively growing one, is the only part of the root capable of curving.

Now, comparing the effects of a transverse current acting directly on a root, and one passing through gelatine, in which a number of roots are growing, a striking difference is apparent. It was remarked in this latter instance, that the curvatures in that portion of the gelatine which was intermediate between the zones extending about 2·5 cms. from either electrode were chiefly to the — electrode whether the medium was acid or alkaline.

An explanation of this may be found by supposing these curvatures to be due chiefly to the travelling ions, and since evidence points to the acid ions being the more effective, the impingement of these on the sides of the roots facing the — electrode would bring about a curvature in that direction.

Another argument which might be put forward to account for this apparent peculiarity is that since roots are normally acid, they might, according to Weber's Law, be more responsive to alkali at first, whereas when the acidity reached a certain intensity its primary greater stimulating action would come into play, and overcome the predominant influence excited by the first traces of alkali. Also roots growing in gelatine would, owing to poor aeration, have more CO_2 in their tissues, and hence might even be more acid than normal.

The curvatures to the + and — electrodes in the immediate vicinity of these electrodes are without doubt due mainly to the direct action of the accumulated acid and alkali in these regions, the ions diffusing to the root in the opposite direction to that in which they are carried by the electric current.

To test how far a curvature was due to the action of an accumulation of ions or to the impingement of travelling ions when the electrodes were directly applied, a few experiments were made, using chloroform to render the protoplasm non-irritable during the time the ions would be travelling through it. At first a saturated solution of chloroform in distilled water was used: the effect of the chloroform lasted so long that no curve was visible within twenty-four hours of stimulation; but a half-saturated solution answered well.

The roots were placed in the liquid for four minutes, stimulated for four minutes, then washed, and after seventeen hours they were compared with a control set, stimulated but not chloroformed. The chloroformed ones curved to a greater extent, and showed more vigorous growth, hence a conclusion might be drawn that this was due to the antagonistic action of the travelling ions being eliminated owing to the non-irritable condition of the roots during the passage of the current, and only the action of the accumulated ions allowed to be effective, since this action comes into play as the effect of the chloroform passes away. An electric current was passed through a dilute salt solution saturated with chloroform, and it was found that the chloroform did not appreciably effect the conductivity of the liquid, so that the presence of chloroform in the root does not alter its conductivity, and hence allow more or less current to pass than through unchloroformed roots. Also some seedlings were chloroformed as above, and laid horizontally, and their feeble curvatures after seventeen hours showed that the chloroform had lowered their geotropic irritability considerably.

The above results show that, although Brunchhorst's facts are correct

as far as they go, the conclusions he draws from them need extension and amplification.

Thus in regard to the − curvatures obtained by him with weak currents acting on roots arranged in water, the stimulatory effect of the travelling ions would preponderate, and of these the acid ones especially, which, impinging first on the sides of the roots facing the − electrode, would produce a curvature in that direction: convection currents, aided by those due to the rotation of the cylinder of water, would prevent any great accumulation of ions at the two electrodes, so there would be no strongly marked acid or alkaline zones as in gelatine, and hence there would be no + curvatures.

This arrangement of growing roots completely immersed in water is not a good one, since they must necessarily lack proper aeration. The curve to the + electrode obtained by using strong currents Brunchhorst considered to be of a traumatropic nature, because his roots, and Elfving's, usually died: but this term is certainly inapplicable to all + curvatures, since it is quite easy to obtain them without injury: though it is quite possible that Brunchhorst's and Elfving's curves were really curves of injury.

Also Spalding [1] has shown that traumatropic curvatures will take place even when growth is arrested for seven or eight days by means of plaster of paris imbedding; whereas an electrically stimulated root, if allowed to remain as long as five days in a cast, will not curve after release.

UNIVERSITY BOTANICAL LABORATORY,
BIRMINGHAM,
May, 1906.

POSTSCRIPT.

After the completion of the foregoing work on the Galvanotropism of Roots it seemed desirable to ascertain whether a powerful magnetic field would also produce stimulatory effects. It is well known that a plant cell is made up of constituents possessing different magnetic permeabilities, and hence, theoretically, a field of sufficiently great power could hardly fail to influence growth in some way or other.

A horseshoe electro-magnet with adjustable pointed pole-pieces was used: each iron core was 7·95 cms. diameter, and its length 32 cms. A current of about 15 amperes, produced from twenty cells (the E.M.F. of each being 2 volts), passed through the coils, against a resistance of 4·15 ohms. The pole-pieces were adjusted so that their distance apart varied from 7·5 cms. to 3·7 cms. in different experiments; and the roots of very young seedlings of *Pisum sativum* and *Vicia Faba* were placed between

[1] Spalding, Annals of Botany, December, 1894.

them in vertical and horizontal positions. The seedlings were either fixed in narrow glass troughs or pinned with brass or silver pins to very thin sections of cork, covered with cotton wool, and were kept moist by frequent supplies of water of suitable temperature. The room was kept at a temperature of about 23° C., but a thermometer inserted among the seedlings during the exposure to magnetic action registered 25° C. The seedlings were arranged as close as possible to the poles without touching, and kept in that position 40 min., 60 min., 2 hrs., 4 hrs., or 6 hrs.; in every experiment light was excluded, and a control was arranged.

When the roots were removed from the magnetic field and examined, some were transferred to a clinostat for twenty-four hours, and others were fixed in chrom-acetic acid, and, after the usual treatment, microtome sections obtained.

These experiments gave only negative results; hence it seems quite conclusive that to produce any influence on the growth of roots a very much more powerful magnetic field would be necessary.

Microscopic examination of the microtome sections fails to give evidence of any change or alteration in the position of the cell contents: also, since in a few experiments, though not in the majority, the geotropic curvatures of the roots placed horizontally just above one pole slightly exceeded those of the roots placed horizontally just below, special attention was directed to the position of the statoliths in the root-cap; but no difference between either set and those of the control roots was to be noted.

Some further experiments were carried out at an electric light station, using the magnetic field produced by a small dynamo through which passed a current of 25 amperes produced from an E.M.F. of 250 volts. The roots were exposed to magnetic action for twenty-five or twenty-nine hours at a temperature of 24° C. Here again no positive results were obtained.

I should like also to make brief reference to the recently published work of Dr. Gustav Gassner (Bot. Zeit., Sept. 1906) on 'Der Galvanotropismus der Wurzeln.'

His method of experimenting was as follows:—A seedling was fixed in a vessel of definite size (20 × 9 × 8·5 cms.) with its root immersed in water or sometimes gelatine: using carbon electrodes, a current of known strength was passed through the water or gelatine and so through the root for periods of time varying from a few seconds to fifteen hours or more: the current density seems to have varied between ·005 and 4·5 M.A. per qcm.

He arrived at the conclusion that Galvanotropism was nothing more than a special case of Traumatropism, and that the + or − direction

of curvature was brought about by differences of current density or by passing the current for varying periods of time, keeping the density constant.

Dr. Gassner does not appear in any of his experiments to have tried the method of applying the electrodes directly to the root and then by varying the points of application changing the direction of the current. His current always traversed the root horizontally; and thus, by using only this one direction he has missed most of the evidence which in my experiments leads to a different interpretation of Galvanotropism.

Moreover, although his work shows that the utmost care has been taken to avoid any source of error, the experiments were conducted without the use of a klinostat—a sufficiently obvious precaution wherever possible, in dealing with root curvatures: also with the exception of the experiments on root-tips, the roots were entirely immersed in water or gelatine—a condition under which they lack aeration in greater or less degree, and in consequence have a diminished power of response.

It seems highly probable, then, that the curvatures obtained by Dr. Gassner were essentially traumatropic, and that, so far as they are concerned, the interpretation of them given in section 7 is a correct one. But it is a far cry from this to the assertion of the identity of Galvanotropism with a special form of Traumatropism ('Auf Grund eingehender Untersuchungen und Vergleiche kam ich zu dem Ergebnis, dass der Galvanotropismus auf Traumatropismus zurückzuführen ist'). Galvanic currents *can* cause injury, in which case the resulting curvature is traumatropic.

My own experiments, however, make it clear that galvanotropic curvatures can be obtained without injury to the root. Similar curvatures can be produced by dissimilar causes; in the one case of injury; in the other as response to a stimulus which no more involves injury than does the shifting of 'statoliths' in response to a changed direction of geotropic action; and that the term traumatropic could in no way be applied to these.

Further, by constantly varying the direction of the current it was proved quite conclusively that the + or − direction of the curve depended on the position of the positive or negative electrodes: moreover, further experiments all pointed to the analogy of these curves with those produced by a chemotropic stimulus.

Jan., 1907.

J. S. B.

The Structure of the Chloroplast considered in Relation to its Function.

BY

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With two Figures in the Text.

OWING to its important rôle in photosynthesis, the structure of the chloroplast has received considerable attention from botanists, and it is interesting to note that a survey of the work done in this direction discloses important points of difference between the various observations made from time to time upon the structure of the chloroplast.

It is clear, however, that the explanation given of the physiological mechanism involved must agree with the results of such structural investigation. Considerations of this kind have led to the publication of the present paper.

HISTORICAL.

Pringsheim,[1] after placing green tissues in water at a temperature of 50°–60° C. for 15–60 minutes, or after exposing them to steam for some time, examined the structure of the chloroplasts microscopically, and came to the conclusion that the ground-substance of the chloroplast is in the form of a hollow sponge-ball, the green colouring matter occurring in the meshes. Having heated the tissues in this manner, he noticed that a colouring matter, which he called hypochlorin, exuded in the form of variously coloured drops.

Kerner[2] stated that the chlorophyll granules exhibited a pellicle-like thickened outer layer, within which was a colourless porous mass built up of reticular or scaffold-like threads.

[1] Pringsheim, Jahrbücher wissensch. Botan., Bd. xii, 1881.

[2] Kerner, Natural History of Plants, p. 371.

The meshes of this sponge-like substance were filled with a green colouring matter dissolved in an oily substance.

Nägeli showed that when chloroplasts were placed in a solution of cane sugar of a certain strength, or in other suitable solutions, they absorbed water, and burst into two valves, which contained the colouring matter.

Timiriazeff[1] repeated Nägeli's experiments and obtained similar results, using chloroplasts of *Phajus* on account of their large size. They remained unaltered in sugar solutions of a certain strength, but split as the solution was diluted. He tried to get a clearer idea of their structure by microscopic examination with red light, and thought he could see the green colouring matter arranged around the outer surface of the chloroplast in small granules. These granules appeared as a peripheral ring of black specks.

As a result of the examination of living chloroplasts Wager[2] came to the conclusion that they consisted of a mass of green granules imbedded in a colourless matrix. In some cases he saw a distinctly fibrillar arrangement of the chlorophyll, and was able to distinguish between a granular structure in the chloroplasts in epistrophe and a fibrillar structure in apostrophe.

His general conclusion was that the chloroplast consisted of a colourless ground substance, of a delicate alveolate structure, in which the chlorophyll was more or less uniformly distributed.

This very scanty historical review[3] suffices to bring out certain points of agreement and disagreement.

It is almost universally accepted that the chloroplasts consist of a colourless ground substance, presumably proteid in nature, and that the chlorophyll is in some way distributed within this substance. Probably it is held in solution in some vegetable fat or oil.

A point upon which there is disagreement is the distribution of the chlorophyll in the granule.

Some observers state that it occurs in a ring at the periphery of the plastid, others that it is diffused uniformly throughout.

It is evident that this question affects the validity of the observations of Nägeli and Timiriazeff upon the splitting of the granule.

As Timiriazeff[4] has previously shown, the distribution of the chlorophyll may be of considerable importance from a physiological point of view. The energy transformation taking place in the chloroplast, apparently requires that the chlorophyll, absorbing the energy, should be distributed

[1] Timiriazeff, Croonian Lecture, Proceedings of Royal Society, vol. lxxii.
[2] Wager, Presidential Address to Section K, British Association Meeting in South Africa, 1905.
[3] For a fuller historical account see Czapek, Biochemie, vol. i, p. 445.
[4] Timiriazeff, loc. cit., p. 457.

in a very thin layer. Then the thinner the layer in which a definite amount of chlorophyll is disposed, the greater the amount of energy set free at any part of that layer. Consequently the efficiency of the chloroplast in bringing about the photolytic decomposition of carbon dioxide must depend on the thickness of the layer.

As some recent work carried out by one of us, in conjunction with Mr. Usher,[1] had confirmed the impression that the thickness of the layer was of importance, it seemed desirable to repeat some of these previous observations, and to see if it were possible to come to an independent conclusion.

The Microscopic Structure of the Chloroplast.

Plants containing relatively large chloroplasts, generally either *Selaginella Martensii*, *S. Kraussiana*, or *Chlorophytum elatum*, were taken, and various attempts were made to elucidate their structure.

In the case of the *Selaginella*, it was difficult even when using Zeiss

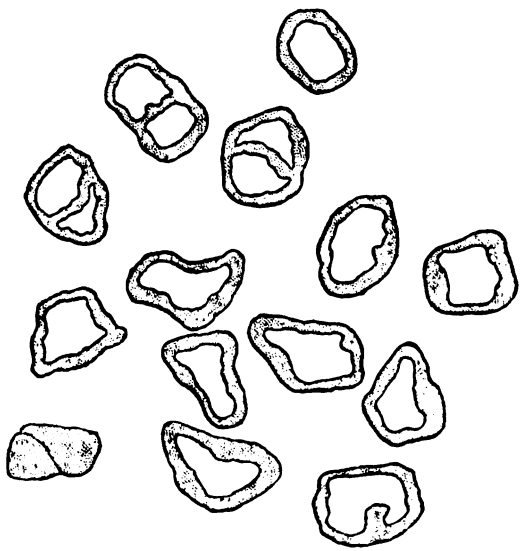

Fig. 1.

apochromatic immersion objectives ($\frac{1}{8}$ and $\frac{1}{12}$) and an achromatic condenser, to discover any signs of heterogeneity of structure; but with *Chlorophytum* such indications were very marked. The chloroplast of the latter plant clearly seemed to consist of a network with chlorophyll in the meshes. Furthermore, in many cases it was possible to obtain optical

[1] Usher and Priestley, Proceedings of the Royal Society B, vol. lxxviii, 1906.

sections of the chloroplasts, which indicated the existence of a distinct peripheral layer to this network. This layer contained the colouring matter.

To obtain chloroplasts for observation in these cases, the leaves of the plant were simply crushed, and some of the green mass was mounted in its own sap.

As the chloroplasts are unusually large in the case of *Selaginella*, the diameter of the largest being about 0·02 mm., an attempt was made to obtain sections of the chloroplast with the fresh material. The sections of the chloroplasts shown in Fig. 1 were obtained in the following way :— The chloroplasts were expresssd in the juice of the plant, mixed with gum and glycerine, frozen, and cut to the thickness of 1 μ by means of a Reichert sliding microtome. A previous attempt to obtain sections from material imbedded in gelatine and frozen proved unsuccessful. The result of this experiment leaves no doubt in our minds, that, in these causes, the chlorophyll is restricted to the outer layer of the chloroplast. This outer layer has a thickness varying approximately from 0·003 mm. to 0·001 mm., as seen in section. Clearly then the actual thickness of the layer must be less.

The green ring thus obtained did not, even under the $\frac{1}{12}$ inch apochromatic, give any clear indications of heterogeneity of structure.

Further evidence was obtained as a result of microtoming the properly fixed and imbedded material. The fixatives used were, Lang's, Merkel's, and Flemming's (stronger solution) fluids, and the sections were afterwards strained on the slide by various reagents, the best results being obtained by the use of gentian violet, and light green.

Material treated in this way gives evidence of a type of structure such as would be seen if a hollow network were cut across.

In the case of *Chlorophytum*, the chloroplasts, after similar treatment, showed the same type of structure. A peripheral network was clearly indicated, even in the case of the fresh material.

The Splitting of the Chloroplasts.

Nägeli was the first to notice the curious appearance obtained in the chloroplast when the usual liquid medium in which it lies is replaced by one of a different osmotic strength.

Timiriazeff has since confirmed these observations, which, however, have not received much attention from other workers upon the subject.

We have also repeated Nägeli's experiments, and have obtained confirmatory results.

The granules, when extracted in their own sap, show no signs of such a crack. But upon adding water, or dilute sugar solution, whilst the chloroplasts still remained under observation, they invariably split, showing, in consequence, a fine dark or light line, which, in a very large percentage

of the chloroplasts, was medianly situated (Fig. 2). The crack, of course, is only visible in certain planes, and as some of the chloroplasts rolled over, it was possible to see it appear and disappear.

A similar crack could be obtained by irrigation with dilute salt solution. But these cracks never appeared in concentrated solutions of either salt or sugar.

When the concentrated salt or sugar solution in which the granules are lying is gradually diluted, at a certain degree of dilution the split rapidly appears in a number of the granules. In most cases it appears simultaneously across the whole width of the granule, but in one or two cases it was seen first at one side, afterwards extending rapidly across the chloroplast.

FIG. 2.

When the split has once appeared, it is impossible to make it disappear by increasing the concentration of the surrounding solution, so that it seems to represent some definite structural change in the granule.

The split is never followed by the complete separation of the two halves of the chloroplast, though in some cases (see Fig. 2) it is impossible to see any connexion still holding the two parts together.

Usually the crack is very regular in its outline, especially in the case of the symmetrical granules of *Chlorophytum*. In the less regular granules of *Selaginella*, the crack was more variable in its nature, and in some cases the granule appeared to have split into three parts.

Some of the split chloroplasts of *Selaginella* were frozen and cut. In these it was possible to discern places where the green layer was discontinuous in the usually uninterrupted rings. This indicated, presumably, the region in which the crack had occurred. All these observations were made on the chloroplasts of *Chlorophytum* and *Selaginella*, but the split can readily be shown in the chloroplasts of many plants, when similarly treated; for example, we have seen it in the *Geranium*, *Coleus*, *Elodea*, *Iris*, and *Amaryllis*.

Effect of Light and Carbon Dioxide on the Splitting.

It was thought that even after expression in sap, the chloroplasts might still be able to form sugar. If this sugar were still retained within the chloroplast there should be a difference of osmotic pressure, comparable to that previously obtained by diluting the surrounding medium, and under the circumstances, a crack might be expected to appear. Leaves of *S. Martensii* and *Chlorophytum* were placed in water in a dark cupboard

for three days; the granules were then extracted under pressure, and upon examination they showed no split. Drops of sap containing the chloroplasts were then mounted on microscope slides in the ordinary way; and all the slides were placed in a moist atmosphere in the greenhouse, some being kept in a sunny position, others in the dark.

It was found that in the slides which had been placed in the sunlight, about 80 per cent. of the chloroplasts had split, whereas a crack had appeared in 5 per cent., at the most, of those kept in the dark.

Fresh slides, prepared in the same way, were then mounted under two bell-jars both in the light, a beaker containing caustic potash being placed in one of the jars, and water in the other.

After having been left for a week, it was found that none of the granules in the atmosphere free from carbon dioxide had split, and would not now do so, even when placed in distilled water. The other set showed the crack in the usual manner.

These experiments seem to indicate that when the granules are isolated in this manner, they are still alive, and capable of synthesizing sugar from carbon dioxide, and water, in the presence of light. The sugar thus formed collects within the granule, while the outside medium remains at its former osmotic strength, and consequently, a split occurs just as when the sap is diluted.

The Effect of Chloroform.

Leaves of *Selaginella* and *Chlorophytum* were killed by treatment with chloroform vapour for two hours. The juice was then expressed as before, and the chloroplasts mounted in distilled water.

In this case it was quite impossible to see any split in the granules. By placing them in a dilute solution of eosin, it was seen that the outer membrane of the plastid had been rendered completely permeable by the action of the poison, the eosin passing into the granule, and colouring it, but not concentrating within it, as it does when the chloroplast is living.

The Nature of the Split.

This split then can be brought about in the chloroplast either by decreasing the concentration of the surrounding sap, or by increasing the concentration of the sugar solution within the granules.

It seems, therefore, to be the result of an internal swelling caused by water passing into the chloroplast, and of the consequent separation of the outer peripheral ring into two halves, the inner colourless stroma not dividing, but holding the two together.

The passage of water inwards may be due to a difference of osmotic pressure on either side of the peripheral layer, which functions as a semi-

permeable membrane, though if this is the case in the living cell, it is difficult to see how the sugar formed by the chloroplast is passed out into the surrounding cytoplasm.

On the other hand, the absorption of water may be due to imbibition by the internal colourless stroma. One would expect neither of these processes to take place after treatment with chloroform.

It has been suggested that this split is really the normal process of division in the chloroplast. It may certainly be an analogous process, but its rare occurrence, except under certain conditions, points to its having been artificially induced in these experiments by definite changes of environment.

Normal division of the chloroplast may be due to similar osmotic differences existing between cytoplasm and chloroplast, but the appearances noted in our experiments, certainly cannot be explained by the assumption that a greater number of chloroplasts were caught in the act of dividing, in one case than in the other.

SUMMARY.

By microscopic investigation of living and fixed material, we have come to the conclusion that in the large chloroplasts of *Chlorophytum elatum*, *Selaginella Martensii*, and *S. Kraussiana*, the chlorophyll is restricted to the peripheral ring of the chloroplast, where it is held in the meshes of a network.

This is in agreement with the views of Timiriazeff concerning the function of chlorophyll in absorbing the radiant energy necessary for the photolytic decomposition of carbon dioxide.

Our confirmation of the observations of Nägeli and Timiriazeff in regard to the splitting of the chloroplasts when placed in a solution of small osmotic strength, is also in agreement with this view of their structure.

Branching in Palms.

BY

H. N. RIDLEY, F.R.S.

With Plates XXXIV–XXXIX.

THE occurrence of abnormal branching of the stem in certain palms has not unfrequently been recorded and figured in a variety of publications, and most of what had been recorded was summed up by Dr. Daniel Morris in his paper 'On the Phenomena concerned in the Production of Forked and Branched Palms' (Journ. Linn. Soc., xxix (1892), p. 281). Having had for eighteen years the opportunity of observing these abnormalities in the Eastern tropics in palms both wild and cultivated, I am enabled to add some descriptions of branching and the production of bulbils in various kinds of palms.

Morris describes the growth of palms as the continuous development of a single monopodial bud, and says that palms have normally an unbranched caudex; but I believe that the greater number of palms are really branched at the base at least, and that cases where there is but one axis produced are rather a departure from the normal.

In many genera we find palms which never produce more than one stem from a single seed, side by side with others which habitually produce lateral buds. Thus we have :—

With one stem.	With several stems.
Caryota urens.	*C. mitis.*
Euterpe oleracea.	*E. stenophylla.*
Areca Catechu.	*A. triandra.*
Raphia Ruffia.	*R. Hookeri.*
Cocos nucifera.	*C. plumosa* (occasionally).

The commonest form of growth in palms is that seen in the soboli-

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ferous species which send out one after another lateral buds from the base, and these ascending become erect stems, so that the whole plant eventually forms a bush. Such are the rattans (*Calamus*, *Plectocomia*, *Zalacca*, and others), *Oncosperma*, *Pinanga*, and others. Then we have a certain number in which some of these shoots grow for a considerable distance, usually under ground, and produce lateral stems at intervals. *Rhapis flabellifera* is an example of this. It throws out slender subterranean suckers to some distance which send up erect leafy stems. In connexion with this plant, commonly cultivated in Singapore, I may remark in passing that the only instance known of its flowering and fruiting is that of a cutting from a rhizome, which on being planted grew out into one poor stem that flowered and fruited several times but never threw up a second stem during the period of observation.

A species of *Pinanga*, perhaps *P. patula*, long cultivated in Singapore, has also subterranean stems which throw up erect leafy branches.

In the two tidal mud palms *Metroxylon* and *Nipa* a large branched rhizome is produced, of a somewhat fleshy nature. This lies half buried in the mud or in drier spots upon the surface of the ground, and produces axillary buds. Some of these ascend and form the tall erect stems in *Metroxylon*, while others, slowly developing, produce the rhizome-branches, from which again ascending stems are developed. This form of palm, although the stems bear pseudo-terminal inflorescences and die after flowering, cannot be called monocarpic in the sense that *Corypha* is a monocarpic palm, as the main stem (the rhizome) does not die, and only the branches are monocarpic.

Morris, in his paper referred to, regards as branched only palms that have divisions of the main stem near the apex, but there is no reason for excluding from the category those that throw out lateral shoots even low down on an ascending stem, which shoots ascend and eventually become often as large as the original stem from which they grew. About twelve years ago, in a clump of *Oncosperma filamentosa* a shoot was thrown out from an erect stem about a foot from the base. It is now (1906) developed into a full-sized erect stem, parallel with the original stem, so that the appearance is that of an equally forked stem, bifurcating a foot from the ground. In many of these developments of lateral buds from the erect stems the growth only lasts for a few years, and the buds die off and disappear, but occasionally, as in the case of this *Oncosperma*, they develop into erect stems.

In the summary of his paper, Morris draws the conclusion that—

1. The branching is due to the injury or destruction of the terminal bud. This may be, and is certainly sometimes the case, in the date palms (*Phoenix*), but is certainly not always or even commonly so in other palms; I never saw an instance in which dichotomy of the

apex occurred, nor one in which the central original bud was crushed out by the development of two lateral ones. In all cases of bifurcation or apparent bifurcation which it has been possible to investigate, one of the branches is a lateral bud, often produced low down on an already tall main stem, and the axillary bud has grown so rapidly that it in time equals the original one in height and thickness.

2. Palms which are usually soboliferous (i. e. produce suckers at the base) are rarely branched at or near the base. Soboliferous palms, however, have a distinct tendency to emit axillary buds well above the base of the main stems, as in the case of the *Oncosperma* above described, and I have seen the same thing in *Dypsis pinnatifrons* and *Chrysalidocarpus lutescens*, both soboliferous palms, as well as in rattans of two genera.

3. No instance appears to be recorded of a monocarpic palm with a branched stem. He defines as a monocarpic palm one which has a terminal inflorescence appearing once only; after the plant has flowered and ripened its fruit it dies. He classes as monocarpic: *Metroxylon*, *Corypha*, *Raphia*, *Caryota*, *Ancistrophyllum*, *Plectocomia*, *Eugeissona*, and possibly *Arenga*. Of these the only ones which are strictly monocarpic are *Corypha* (all species), *Raphia Ruffia*, and perhaps some species of *Metroxylon*.

Plectocomia and *Eugeissona* are very long-lived plants, of which the branches flower in turn and die, but the plant does not. The *Caryotidae* flower in a peculiar way. The stem, when adult, commences to produce flower-buds through the leaf-sheaths from the top downwards, often alternately male and female, till the last bud close to the ground appears, when the whole stem dies. In the case of *Arenga saccharifera* and *A. Listeri* only one stem is produced, so that the whole plant dies after it has finished flowering. The same thing occurs in all species of *Caryota* except *C. mitis*, which produces five stems or more, but when all have done flowering the whole plant dies. In this species the stems are not produced all at once, but one after the other. I have never seen any of these plants producing axillary buds except from the extreme base.

Palms which start throwing out axillary buds on the erect stems seem to have a tendency to continue throwing out buds from the adventitious stem, as may be seen in *Cocos nucifera* and in *Chrysalidocarpus*. In many cases observed the axillary buds are never well developed, and perish soon.

Plectocomia constantly throws out buds, sometimes from each of the lower nodes, but they rarely, if ever, grow more than a few inches.

In some cases the plants which produce these lateral buds are weak and more or less diseased or injured by insects, but this is by no means always the case. However, I do not remember ever to have come across any branched palms in a wild state except *Plectocomia* and some other rattans.

The following is a list of all the branching palms known to me, including those in Morris's list:—

Areca Catechu.
Rhopalostylis sapida.
Dictyosperma album.
Oncosperma filamentosa.
Dypsis pinnatifrons.
Oreodoxa regia.
Leopoldinia pulchra.
Chrysalidocarpus lutescens.
Phoenix, most species.
Nannorrhops Ritchieana.
Hyphaene thebaica, H. coriacea, H. Petersiana, normally branched.
Borassus flabelliformis.
Cocos nucifera. The commonest recorded.
Livistona humilis.
Calamus leptospadix.
Plectocomia Griffithii.

Of these it may be noticed that the greater number were cultivated or planted away from their original home, viz. *Areca, Dictyosperma* (in one case at least), *Phoenix dactylifera, Cocos, Chrysalidocarpus, Oncosperma filamentosa, Dypsis, Borassus* (in most instances), and *Calamus leptospadix*; and the best shoot-bearing *Plectocomia* I have seen in an abnormal position in open ground in the Botanic Gardens. While of the others, *Nannorrhops, Rhopalostylis, Hyphaene, Borassus, Livistona humilis* occur on the furthest limits of the palm regions, where palms are very scanty, and where the climate appears generally to be dry and the soil rocky or sandy, viz. Afghanistan, New Zealand, Africa, and North Australia.

Areca Catechu (Pl. XXXIV). Among the curiosities brought to the recent Agri-Horticultural Exhibition in Singapore was a very remarkable specimen of a betel-nut palm (*Areca Catechu*) from Pulau Kukub in Johore, sent by the late Mohammed Alsagoff. The tree had been cut down, and shows a lower portion about 3 feet long and 6 inches in diameter, of nine internodes, of which the upper one is somewhat dilated and flattened. Above this is a broad mass of roots, 16 inches wide and about a foot long, from which spring five almost parallel erect branches, 24 feet long to the leaves. All the branches are approximately equal in size, and bear well-developed leaves and inflorescences. At 15 feet from the mass of roots one of them emits a lateral branch, about 6 feet long, bearing leaves. The branching of the betel-nut palm is, I believe, very rare; I have only seen one other instance, that of a specimen shown at a Penang exhibition many

years ago, which bifurcated rather high up, and produced two strong equal stems. This specimen was sent to the British Museum.

In the Johore specimen it appears from the slight flattening of the stem below the mass of roots that this branching is due to fasciation and not to simple axillary buds, as is commonly seen in coco-nut palms, where the branches are often at least distinctly alternate or in simple pairs. Morris, in his paper on branched and forked palms, mentions a few instances of branched betel-nuts, one dichotomously branched at Trevandrum (South India) and one simply branched with two stems at Cayenne. One in Tangire described by Mr. Sinclair more resembles the Johore specimen. It had been attacked by a disease called Band, which had killed many trees in the vicinity, and its top nearly died away, and was replaced by fifteen to eighteen tops growing in a flat close bundle, so that they could not be counted separately without climbing the tree.

Chrysalidocarpus lutescens (Pl. XXXV) is a Madagascar palm often cultivated here. It forms large tufts branching from the roots and attaining a height of 12 feet or more. In one clump in the Botanic Gardens in 1894 I found a number of branching stems. Axillary buds were produced at various heights from the ground. They were emitted at the nodes, and usually slender here, at first more or less horizontal, then ascending and dilating so as often, in early stages, to be fusiform in outline. One emitted roots from its lower nodes, which did not reach the ground. There were often two of these branches on the stem, and not rarely they again branched. The terminal bud of the main stem was uninjured, and the branches were often much below it, and much shorter. Most of these branches died away after a time, but one or two still remain on the clump (1906), and have grown to be almost equal to the main stem in height, so that the stem appears to have bifurcated. The clump which produced most of these shoots is somewhat weakly, compared with others in the garden, and I have noticed the same tendency to throw out side-shoots from the axils in ill-nourished plants of *Dypsis madagascariensis*. The sketches in Plate XXXV are all from the same clump of the palm.

Cocos nucifera. There are many more instances recorded of branching in coco-nut palms than in any other species of palms. This is doubtless due in great measure to the large number of plants in cultivation and to their being very conspicuous and noticeable when branched. Still, in proportion to the number of trees in cultivation, the percentage of branched trees is not large. 'The characteristic feature in branched coco-nuts is the simple fork,' says Morris, and he goes on to suggest that the cause may be due to development of a lateral bud, as in *Hyphaene*, or be consequent upon injury to the terminal bud; 'in the latter case the terminal bud

is destroyed by insects or through some mechanical injury.' 'Two axillary buds inserted immediately below would grow into branches.' This latter suggestion is, I think, an extremely doubtful one. Of the hundreds of trees I have seen attacked by coco-nut beetles so that the terminal buds were destroyed, I have never seen one in which any lateral buds were extruded. Furthermore, when a coco-nut commences to branch, its branches usually continue to branch again, nor indeed does it show any signs of injury by insects or in any other way. In Perseverance Estate in Singapore there was formerly a very remarkable coco-nut tree (Pl. XXXVI), which branched in a somewhat abnormal manner. From a main single stem were emitted three branches. The central one, probably the original main bud, branched again, and both branches died. The other two grew tall, and each branched in a bifurcating manner. One branch died, the other went on growing and branched again. Again one branch died and the other continued to develop, and this happened alternately, every alternate branch dying. In fact, the growth is in this case cymose.

This tree, I was told, had never fruited, and showed no signs of having been attacked by insects or otherwise injured. In all cases of simply forked palms I have seen, the forking is caused by the development of an axillary bud well below the main terminal bud, which it usually catches up in growth some years later, and being then equal in size or nearly so, and parallel to it, suggests that the top has at some time branched, either by dichotomy of the bud, or by two equal buds starting just below the main bud and squeezing it out; I do not, however, see any evidence for either of these theories, and in palms of which I have been able to observe the origin and growth of the two branches neither of these things has occurred.

A bulbilliferous coco-nut (Pl. XXXVII, XXXVIII). For an account, photograph, sketches, and bulbil I am indebted to Mr. R. Scott, who found the tree at Lumut, in the Dindings. The tree, he says, is reported to be ten years old (in 1903), and grows in a Chinaman's coco-nut plantation at Pundut, about five miles from Lumut. It resembles an ordinary coco-nut, except that the foliage is thicker, and has never borne fruit. About five years previously, when it should have started bearing, it put out deflexed shoots instead of flower spathes. These shoots grow for 4 or 5 feet long, the leaves being 2 inches through; when they are as big as this the whole shoot seems to get too heavy, and drops off, and fresh ones appear. The whole appearance of the shoot is like an ordinary young coco-nut, and the centre is formed of a jointed stem about 30 inches long. Bulbils of this nature are not rare in other Monocotyledons, and one might compare them to those of *Globba*, but I do not know of a similar case in a palm. Morris, in describing the branching of *Nannorrhops*, considers that the shoots are replacing the inflorescences, and alludes to a similar occur-

rence in *Phoenix sylvestris*, and perhaps in the Lumut coco-nut we have a parallel case.

Plectocomia is an enormous rattan which, like others of the climbing Calameae, throws up from its base a number of shoots. Of these some, if not all, develop into extremely long branches, which with the aid of their flagella, armed with hooks, climb to a great height on forest trees. These branches in *Plectocomia* are comparatively slender at the base and thicken upwards. In the lower part of the branch, which often lies on the ground, roots are emitted from the nodes, and are practically aerial roots, for, except at the extreme base, they never reach the ground, but run along the branch downwards. It is by no means uncommon to find also shoots emitted from the nodes, and one plant in the Botanic Gardens has produced a shoot from every node near the base. These shoots never develop into branches, but remain quite small, and usually perish with the long climbing branch, which dies completely after flowering.

Calamus leptospadix, a slender rattan from India, grown in the Botanic Gardens at Singapore, not rarely produces (especially when the long climbing stem touches the ground) shoots at the nodes, by which the plant can be propagated.

This layering of a rattan branch is not very uncommon in the jungles, especially where the rattan has fallen across a wild beast track, and elephants, rhinoceros, and such large animals in walking along the track constantly step on the prostrate rattan. The rattan emits roots from its nodes, and then develops a tuft of shoots, which may in time become transformed into a fresh bush.

From these Calami can be obtained the so-called 'reversed rattans,' in which the leaf-sheaths apparently point towards instead of away from the dilated root-bearing portion—very puzzling to explain till it is realized that the thickened head of the cane is not the real base of the rattan, but the thickened portion, which has been layered at some distance from the original root of the plant.

Calamus sp., (Pl. XXXIX). This elegant little dwarf rattan is abundant on Matang mountain in Sarawak. Its stem is a few feet long only, and it hardly climbs. The inflorescence is very lax, and 4 or 5 feet long, with much branched panicles of slender branchlets in the male and fewer and often simple branchlets in the female plant. The inflorescences hanging down usually rest on or close to the ground of the banks in which it grows, and where they do so bulbils are produced from the axils of the sheathing bracts in place of a branch of the inflorescence or quite replacing it. These bulbils take root and develop into fresh plants. This seems to be commoner in the female inflorescence, which is shorter than the male, and generally the bulbils are produced towards the end of the inflorescence. This is not a casual abnormality, but seems to be regular; most female

plants I have seen at least doing so. The plant fruits also, but I have not come across any fully ripe fruit. Judging, however, from the appearance of the plants, especially on the top of Matang hill, this method of reproduction is the commonest. I am quite unable to identify this plant with any of Beccari's descriptions, which in many cases are quite insufficient for purposes of identification, but as it is improbable that he overlooked during his stay on Matang so abundant a plant as this, it is probably one of his species.

EXPLANATION OF PLATES XXXIV–XXXIX.

Illustrating Mr. Ridley's paper on Branching in Palms.

Pl. XXXIV. *Areca Catechu.*

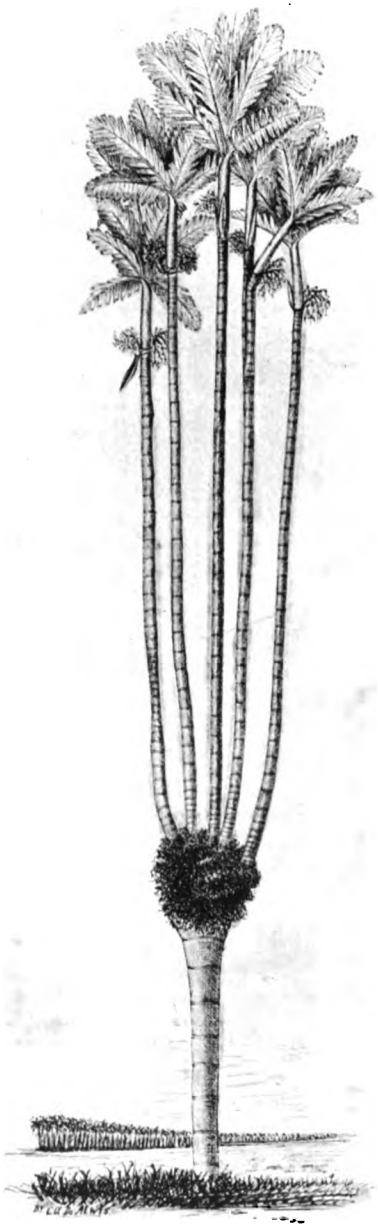
Pl. XXXV. *Chrysalidocarpus lutescens.* *A, B, D* drawn in 1894; *C* in 1906. *E* is *D* as it now appears in 1906. All from the same clump.

Pl. XXXVI. Coco-nut, branching, from Perseverance Estate, Singapore.

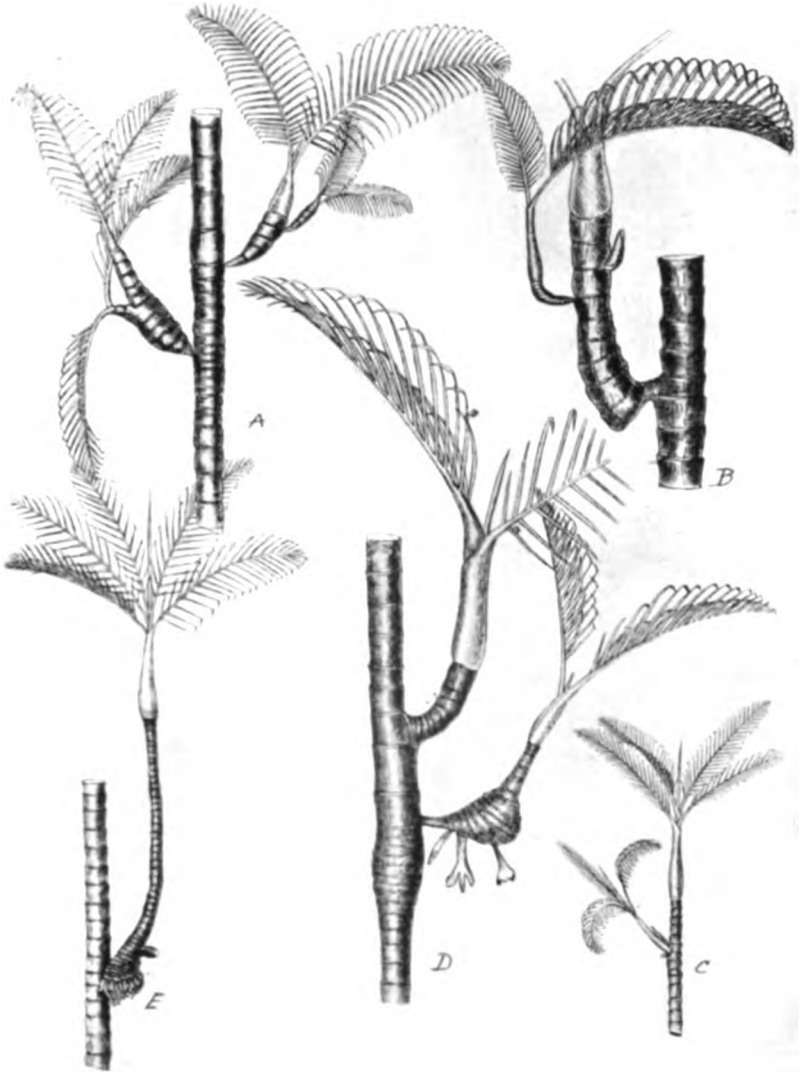
Pl. XXXVII. Bulbilliferous coco-nut. Complete tree.

Pl. XXXVIII. A bulbil.

Pl. XXXIX. *Calamus sp.* Sarawak. Portion of inflorescence with bulbils.



RIDLEY—BRANCHING OF PALMS.



RIDLEY—BRANCHING OF PALMS.

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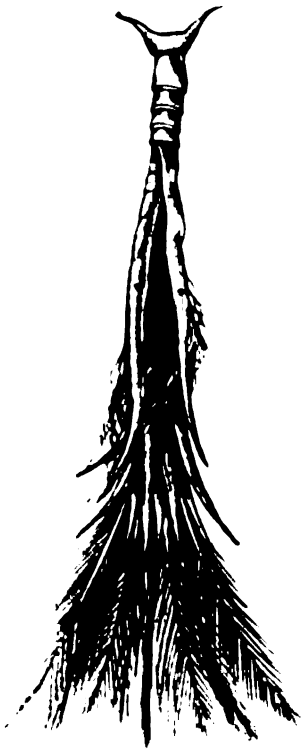

RIDLEY—BRANCHING OF PALMS.

RIDLEY—BRANCHING OF PALMS.

Studies on the Occurrence and Reproduction of British Freshwater Algae in Nature.

I. Preliminary Observations on Spirogyra.

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With Eleven Figures in the Text.

A. INTRODUCTORY.

IN the course of extensive investigation of periodical samples of Algae from various ponds, situated mainly in the south of England, a considerable number of facts regarding the occurrence and reproduction of *Spirogyra* have come to light. Certain data dealing with the biology of this genus have already been published,[1] but we believe that the subject has not yet been approached from exactly the same point of view as that adopted in the present paper. A general preliminary account of the results so far obtained may thus be of some value. It is proposed in subsequent papers of this series to deal with other genera of freshwater Algae in the same way.

The materials on which our observations are based have been collected by the method already described in some detail by one of us,[2] and it

[1] See especially Messrs. W. and G. S. West, Observations on the Conjugatae. Annals of Botany, vol. xii, No. xlv, 1898, p. 29 et seq. J. Comère, Observations sur la périodicité du développement de la flore algologique dans la région toulousaine. Bull. Soc. Bot. de France, t. liii, 1906, p. 390 et seq. G. Klebs, Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen. Jena, 1896, p. 229 et seq.

[2] Cf. Fritsch, Problems in Aquatic Biology, &c. New Phytologist, vol. v, No. 7, 1906, p. 149 et seq.

[Annals of Botany, Vol. XXI. No. LXXXIII. July, 1907.]

is unnecessary to say much more about it here. We are glad, however, to have this opportunity of expressing our sense of obligation to a number of botanists, who have with such kind perseverance and care considerably furthered these investigations. We are fully aware of the objections that can be raised against observations based on the collection of isolated monthly or fortnightly samples by individuals who are not in direct touch with the work. But apart from the fact that most of those who have collected for us have evidently done so conscientiously, we may point out that in dealing with small pieces of water like those to which we have confined our attention, it is very unlikely that a form, which is at all common, will have escaped observation; and we have been careful not to draw many conclusions as to relative amounts of an Alga present without the existence of overwhelming evidence. Much labour has been bestowed on a thorough examination of each sample of Algae, particularly where a negative conclusion was concerned.

The conditions, which lead to and modify the process of reproduction in *Spirogyra* (and other Algae) have been investigated by Klebs, and are published in his 'Bedingungen der Fortpflanzung, etc.' (Jena, 1896). Klebs's results are of great interest, and we shall have frequent occasion to refer to them in recounting our own observations, but there is one objection to which a large number of his experiments are open. It is doubtless of some value to know how diverse reagents and changes in external factors affect the reproduction of an algal genus, but unless they are such as are realized in nature, they do not contribute materially towards an understanding of its biology. To our thinking work of this kind is best started from the other side; that is to say, we should commence by undertaking careful observations on the Alga in nature, and endeavour to correlate any changes it shows with variations in the natural conditions of its habitat. The Alga in this method of investigation is left to react under the play of nature's forces, and it is the work of the investigator to interpret such reaction in terms of external factors. When that is accomplished we must resort to experiment, which must ultimately settle whether the inference from direct observation is correct or not.

B. The Occurrence of Spirogyra in Nature.

In dealing with the occurrence of *Spirogyra* in nature we must distinguish between a vernal and an autumnal phase, which are more or less sharply marked. It is in the vernal phase that reproduction mainly takes place, although, as we shall see, this is not without exception. Some species of *Spirogyra* (e.g. *S. varians* (Hass.), Kütz., *S. quadrata* (Hass.), Petit) would appear to be (ordinarily) confined to the vernal phase and not to reappear again in the autumn, while others (e.g. *S. rivularis*, Rabh., *S. affinis* (Hass.), Petit, *S. jugalis* (Dillw.), Kütz.) are present both in spring

and autumn. Between the vernal and the autumnal phase, however, there is always a very marked decrease in quantity, amounting in very many cases to complete disappearance (in July and August, cf. *S. affinis*, *S. jugalis*, *S. rivularis*). There is some evidence also to show that between the autumnal phase and the ensuing vernal phase some species of *Spirogyra* again practically disappear, being absent in midwinter (cf. *S. jugalis* (Dillw.), Kütz., *S. Weberi*, Kütz., *S. rivularis*, Rabh., in 1905). Since zygospores (except in a few cases) are not formed during the autumnal phase, the reappearance of the Alga in the following spring must take place at the expense of the zygospores formed in the preceding vernal phase. The Alga never appears in autumn in such quantity as to warrant the supposition that all the zygospores formed in the previous spring have germinated; in all probability the conditions which lead to the autumnal appearance of these *Spirogyras* are only sufficient to stimulate a small number of zygospores to germination, and the main mass of them remains dormant till the spring.[1] We shall return to this subject below.

Before passing on to further considerations we may briefly discuss some of the data in the literature bearing on this subject. Comère (loc. cit., p. 405) records the occurrence of Zygnemaceae in the waters examined by him as follows: In the 'eaux stagnantes' they are found in his second vernal, aestival, and autumnal periods (i. e. from the middle of April to the first frosts), while in the 'milieux passagers' they are present only in the first and second vernal periods (i. e. from the end of February to the end of June).[2] While we have been unable to recognize any marked differences in the occurrence of *Spirogyra* in permanent waters and in those which dry up in summer, Comère's observations agree with ours in the prevalence of this genus in the spring. The waters examined by Comère do not apparently show any marked decrease of *Spirogyra* in midsummer, while its absence in winter is much more marked. Petit[3] in his valuable treatise on the *Spirogyras* of the neighbourhood of Paris mentions March—July as the period of occurrence of most of the species described, although many of them have a much more limited period; *S. orthospira* (Naeg.), Kütz., *S. bellis* (Hass.), Cleve, and *S. orbicularis* (Hass.), Kütz., are the only species found by him also in the autumn. Petit's observations are particularly valuable, as they are the results of investigations extending over many years. According to Klebs (loc. cit., p. 229), species of *Spirogyra* are found 'zu

[1] It is not quite impossible that the disappearance of *Spirogyra* in summer and midwinter may be only apparent, and that a certain number of filaments may sink to the bottom and remain there in a dormant condition. In view of the fact that our knowledge of algal reproduction is so scanty, it is just worth while keeping this in mind, although there are no data to support it.

[2] The pieces of water with which we are concerned in the present paper belong only to Comère's 'eaux stagnantes' and 'milieux passagers.'

[3] Paul Petit, *Spirogyra* des environs de Paris. Paris, 1880, 39 pp. Unfortunately, Petit does not definitely state whether the months mentioned by him after each species refer to period of occurrence or period of reproduction, but it seems probable that they refer to the former.

allen Jahreszeiten in Sümpfen und Teichen,' although his own subsequent remarks do not point to an occurrence at *all* times of the year. Regarding *S. inflata*, Vauch. (p. 233), he states that he found it (in the sterile condition) in spring, 1894 (apparently present already in February), and that the first indications of conjugation were observed at the beginning of May. *S. Weberi*, Kütz. (p. 241), appeared regularly in a pond in the Botanic Gardens at Basle in March and attained an abundant development in the course of April; at the end of April or the beginning of May conjugation took place, after which the Alga disappeared. Klebs found this species again in the autumn, and remarks upon it as follows: 'Erst im Herbst, sei es aus überlebenden, einzelnen Fäden, sei es aus Zygoten, entwickelt sich die Alge noch einmal, kommt aber nicht mehr zur Konjugation.' This is in complete accord with the observations we have made on some species of *Spirogyra*, although in the case of *S. Weberi* our data testifying to an autumnal occurrence are rather meagre. These few statements quoted from the literature indicate that the general occurrence of *Spirogyra* is much as we have described it in the preceding paragraph, but that minor modifications of the ordinary scheme are frequent (as indeed our own observations show). It is not at all likely that the periodicity of *Spirogyra* (or of any other Alga) will be quite the same even in the same latitudes as numerous modifying climatic factors must come into play.

The different species of *Spirogyra* certainly as a rule attain their maximum development in the vernal phase. The autumnal phase is on the whole quite subsidiary to the vernal phase—a fact which is already evidenced by the lack of reproduction in the autumn. The reappearance of species of *Spirogyra* in the autumn probably depends on the realization of certain external conditions which stimulate a certain number of the zygospores to germination. In the absence of the necessary conditions the autumnal phase may be far less evident, or even completely suppressed, and species which are ordinarily present both in spring and autumn may appear to have a vernal phase only (cf. *S. jugalis*, present in Abbot's Pool in autumn, 1905, but absent in autumn, 1906; see also *S. affinis*). On the other hand, it is quite possible that those species, which we have been led to regard as purely vernal ones (e.g. *S. varians*), do under exceptional circumstances exhibit an autumnal phase as well. We have no data at present in support of this latter view, but they may be forthcoming in the course of further investigation. All species of *Spirogyra* would then have the tendency to be present both in spring and autumn, but in the case of some of them, the necessary conditions for an autumnal appearance would only very rarely be realized. The causes for the disappearance of *Spirogyras* after the vernal phase will probably be found in some or all of the factors connected with the advent of summer. These factors may be enumerated as follows:—

(*a*) The increase in the intensity and duration of the light.

(*b*) The increase in the temperature of the water and the consequent diminution in the amount of dissolved gases in the water.

(*c*) The gradual concentration of the salts dissolved in the water owing to the heat, and lack of rainfall in a normal summer.

(*d*) ? The increase in the amount of the higher (Phanerogamic) vegetation present.

At the end of a normal summer all these factors undergo modification in the reverse direction, and in this way conditions may become favourable for the reappearance of *Spirogyra*. We may point out, however, that certain definite combinations of these external factors are probably necessary for the reappearance of any given species, and that these combinations will vary for each individual species. From observations made last autumn it would seem as though dilution of the water back to its ordinary degree of concentration were one of the essential factors for an autumnal phase, but that if this dilution is delayed too long it may take place at a time when the average daily temperature or light-intensity is not high enough to allow of the appearance of *Spirogyra*. Thus in the autumn of last year (1906), in which after a very dry and hot summer rain only set in at a late date, *Spirogyras* were practically wanting in all the ponds examined, whilst in 1905, in which the rain commenced early and was rather equally distributed over the autumn months, many of the *Spirogyras* showed a very well-marked autumnal phase (cf. the table). In the case of one pond (Barton's pond at Harpenden), in which *Spirogyras* were very common in the spring, the water in 1906 only attained its ordinary level after the summer shrinkage at the end of October, i. e. at a time when the average daily temperature was 10·9° C. (on November 7th the average for the previous fortnight was only 6·8° C.); there was practically no autumnal *Spirogyra*.

The above suggestion that the occurrence and especially the extent of the autumnal phase depend on certain combinations of external factors is supported by a number of data, which may be briefly referred to; they are mainly derived from Abbot's Pool, near Bristol, from which we have three years' consecutive observations. *S. rivularis* was fairly abundant in this pond in the autumn of 1904, but much rarer, though always present in some quantity, in the autumn of 1905 and 1906. *S. jugalis* was quite a common form in the autumn of 1905, but absent at this time of the year both in 1904 and 1906. The special conditions, which led to the non-development of *S. jugalis* in autumn, 1904 and 1906, did not, therefore cause the disappearance of *S. rivularis*, which even flourished in 1904.

The disappearance of *Spirogyras* in midwinter between the autumnal and the vernal phase, which is not quite certainly established, can only be related to temperature and light-conditions. It would be premature to discuss the matter further at the present moment.

The ordinary curve of frequency of *Spirogyra* would thus either be a single one (with vernal phase only) or a double one (with both vernal and autumnal phase) with two maxima, one of which (in spring) would be much higher than the other (in autumn). *Spirogyras* are, however, occasionally found flourishing at other times of the year, and we must now notice a few cases of this kind. In the aquatics' tank near the Jodrell Laboratory at Kew, *S. crassa* is present in quantity practically all the year round, although it has not been found reproducing; *S. condensata* and *S. longata*, however, are ordinarily only common in the same piece of water from June to August. This tank belongs to Comère's 'milieux mixtes,' and the period of occurrence of the two *Spirogyras* last-named roughly agrees with Comère's observations on such pieces of water (loc. cit., p. 405).[1] In a pond at Telscombe, near Newhaven, *S. insignis* (Hass.), Kütz., was present in very large amount (but did not reproduce), in December, 1902, and January, 1903, although it disappeared totally after that, and did not occur again in the following winter.[2] *S. affinis* (Hass.), Petit, and *S. cataeniformis* (Hass.), Kütz., were very abundant from September to December, 1904, in the same pond (cf. table). *S. communis* (Hass.), Kütz., is frequently very abundant in midwinter in one of the ponds on Sheen Common, Richmond. We cannot do more than merely mention these cases, which no doubt any one of our readers could multiply. Further observation and experiment will no doubt bring an adequate explanation of these apparently abnormal phenomena.

It still remains to consider a few examples of the reverse state of affairs, i. e. absence of a species of *Spirogyra* at a time when one would expect it to be present. One of the most striking cases of this kind is the complete absence of all species of *Spirogyra* in the first half of 1904[3] in Abbot's Pool, although in 1905, 1906, and 1907 they were present in great amount. We are indebted to Dr. H. R. Mill for data as to the rainfall in the early part of 1904 and in the latter half of 1903. In October, 1903, the rainfall at Clifton was very much in excess of the average (= 4·01 in. for the last thirty years), viz. 8·03 in.; December also had a high rainfall, viz. 3·13 in. The total rainfall from September to December, 1903, amounted to 16·4 in., as against 7·35 in. in 1904, 7·51 in. in 1905, and 11·13 in. in 1906. With reference to the sunshine, Dr. Mill writes: 'I cannot speak positively about the sunshine, as I have no statistics of it, but I think you may safely take it that the last three months of 1903, and the earlier months of 1904, were decidedly deficient in sunshine.' There is thus good evidence to show

[1] Cf. Fritsch, Algological Notes. IV. Remarks on the Periodical Development of the Algae in the artificial waters at Kew. Annals of Botany, vol. xvii, 1903, p. 277; cf. also occurrence of *Spirogyra* in the lake at Kew.

[2] Fritsch, Problems in Aquatic Biology, &c., loc. cit., pp. 164, 165.

[3] Unfortunately, we have no samples from this pond before January, 1904, so that we are unable to say how long previously this state of affairs had obtained.

that prior to the abnormal absence of *Spirogyra* in Abbot's Pool in the spring of 1904 the state of the weather was not at all normal (very excessive rainfall and lack of sunshine). That these conditions have something to do with the vernal appearance of *Spirogyra* is also exemplified by the remarkably late appearance of this genus in Abbot's Pool (and other ponds) in the spring of this year (1907). Ordinarily, *Spirogyra* is quite abundant already in March; this year, however, it did not occur in any amount before April (cf. the table).[1] As above shown, the rainfall in the autumn of 1906, although not as great as in 1903, was considerably above that of 1904 and 1905; this may again have something to do with the late appearance of *Spirogyra* this spring. There has, however, been a general dearth of *Spirogyra*[2] this spring, and we should not like it to be imagined that we consider the rainfall of the previous autumn solely responsible. The last year had a very long and hot summer, during which many of the smaller pieces of water dried up, and we have already pointed out that the ordinary water-level was probably regained too late for the proper development of an autumnal phase in any *Spirogyra*; this may have acted as a check on these forms, which may be connected with their late appearance this spring. The spring of this year has moreover also been abnormal, and may be partly accountable.

C. The Reproduction of Spirogyra in Nature.

The fact that in most cases the species of *Spirogyra* reproduce during the vernal phase is a very marked feature in the biology of the genus (cf. the table on p. 436). The conditions which lead to reproduction at this time of the year must be very complicated ones, and we may quote the following examples in illustration of this. In the case of Abbot's Pool, near Bristol, *S. varians* (Hass.), Kütz., has been found reproducing abundantly in the spring of 1905, 1906, and 1907; *S. Weberi*, Kütz., and *S. affinis* (Hass.), Petit, although present in all three years, formed zygospores only in 1906; *S. nitida* (Dillw.), Link, was present both in 1905 and 1906, but reproduced only in 1905; while *S. neglecta* (Hass.), Kütz., formed zygospores in both years. In all three years *S. varians* was found with zygospores in two other ponds during the vernal phase (cf. the table). In the case of this species, therefore, the conditions necessary for reproduction appear to be realized every spring; while in the case of the other species mentioned above, the factors which lead to conjugation only occasionally obtain. Similar observations were made on the *Spirogyras* in Barton's Pond, near Harpenden; *S. tenuissima* (Hass.), Kütz., was reproductive in both 1906

[1] Reproduction also set in considerably later than usual.

[2] In some ponds, which ordinarily have a good deal of *Spirogyra* in spring, it has scarcely put in an appearance up to the time of writing (June 3), e.g. Hendon Pond.

and 1907; *S. quadrata* (Hass.), Petit, and *S. cataeniformis* (Hass.), Kütz., formed zygospores only in 1906, *S. Hassallii* (Jenn.), Petit, only in 1907.

Although the period of reproduction may be roughly limited to April-June, reproduction takes place at different times in the individual species during this period (cf. the table). Thus in Barton's Pond a considerable amount of sterile *Spirogyra* was present on April 9, 1906; on April 21, *S. quadrata* (Hass.), Petit, and *S. tenuissima* (Hass.), Kütz., were observed with zygospores, while *S. cataeniformis* (Hass.), Kütz., was beginning to conjugate; on May 5 matters were unaltered, and *S. cataeniformis* was only observed with zygospores on May 19. By June 18 all *Spirogyras* had disappeared. Similarly, in Abbot's Pool in 1905, *S. varians* and *S. jugalis* were found in the reproductive condition in April; while *S. neglecta* and *S. nitida*, although previously present, did not begin to reproduce before May. In some species the period of reproduction is remarkably long (e. g. *S. affinis* (Hass.), Petit, *S. neglecta* (Hass.), Kütz.), in others short (e. g. *S. varians* (Hass.), Kütz., *S. Weberi*, Kütz., *S. jugalis* (Dillw.), Kütz.).

As a rule, if the same species is found in two or more ponds, and is reproductive in one of them, it is found to reproduce in all of them, though not always at exactly the same time (e. g. *S. varians*, *S. neglecta* var. *ternata*, *S. quadrata*, *S. jugalis* in 1905); this is, however, not without exception (cf. *S. Weberi*, *S. jugalis* in 1906). In the case of some species (e. g. *S. varians*, *S. tenuissima*, *S. condensata*, *S. affinis*) the conditions causing reproduction affect nearly all the filaments, and with the formation of zygospores the species disappears; in other cases, however (e. g. *S. neglecta* var. *ternata*, *S. nitida*, *S. Weberi*), by no means all the filaments become involved in conjugation, and these sterile filaments may persist for some time after zygospore-formation. This phenomenon is no doubt also dependent on the degree of development of the external factors influencing reproduction in *Spirogyra*, and one and the same species may in some cases disappear completely after reproduction, in other cases still persist for a time (cf. *S. affinis*, *S. neglecta*, &c.). Another feature illustrating the varying effect of external conditions on reproduction in *Spirogyra*, is the occasional conjugation of a species without the process coming to an end (i. e. without formation of zygospores), cf. *S. jugalis* (Dillw.), Kütz., in Abbot's Pool in 1905, and at Tiltham's Pond in 1906.

The facts detailed in the preceding paragraphs suffice to show the complexity of the conditions influencing vernal reproduction in *Spirogyra*. The prevalent occurrence of reproduction in the vernal phase may be due to an inherent tendency, or to certain combinations of external conditions, which occur more or less regularly every spring (seasonal factors). If vernal reproduction is the result of inherent tendency, then it is difficult to understand why a species reproduces in the spring of one year and not (although present) in the spring of another year (cf. the data given above); moreover,

the occasional reproduction of *Spirogyra* at other times of the year is incomprehensible. With regard to the former point we cannot deny the possibility of some of the *Spirogyras* being biennial as far as reproduction is concerned; one or two years' further observations will show whether there is any truth in this. But from all we know about algal reproduction it seems very unlikely, and it is far more probable that reproduction in *Spirogyra* depends on the presence of certain combinations of factors, which probably differ for each individual species. The most important changes that take place in spring are on the whole the same as those which have been enumerated on p. 427; it does not seem likely, however, that there is any marked annual concentration or dilution of the water during the early part of the vernal phase (March and April), although this factor will in many years come into play during May and June. All the factors concerned, it will be noted, undergo a gradual intensification as the summer approaches, and this intensification reaches its maximum somewhere during the summer months, after which there is again a gradual diminution. At some time in the autumn, therefore, each factor must be present in the same degree as in spring, and it might be argued that autumnal reproduction should for this reason be as common as vernal reproduction. In the case of the autumn, however, each factor is undergoing change in the reverse direction (intensity and duration of light decreasing, temperature decreasing, &c.) to that which obtains in the spring, and this difference probably accounts for the absence of autumnal reproduction—even though combinations of factors, similar to those found in spring, must sometimes occur. The direction of change of a factor or of a group of factors, is no doubt of great importance, and it probably makes all the difference whether, for instance, an Alga after being exposed to a low temperature is subjected to a higher one, or vice versa. The response in the two cases may be quite different.

The reproduction of the different species of *Spirogyra* in the vernal phase is thus most probably dependent on the seasonal occurrence of certain external conditions, particularly on an increase in intensity of the factors liable to change in spring. For each particular species certain intensities of the different factors will probably be necessary, and it depends on the realization of these intensities as to whether the given species will reproduce or not. That these intensities vary for different species, is shown by the fact that in the same pond different *Spirogyras* begin to reproduce at different times during the vernal phase, and that the same species reproduces at different times in different ponds and in different years (cf. the table). The general *modus operandi* of these factors is illustrated by Klebs's work. He has shown (loc. cit., pp. 233, 237, and 241) that in the case of a number of species of *Spirogyra* zygospore-formation can be induced by placing the Alga in water in bright sunlight for a few days. If we substitute a weak nutritive solution for the water, it will, unless very dilute, absolutely work

against the effect of strong sunlight, and no conjugation will take place. Temperature has very little effect on the process except to accelerate it. We have no data as to the possible effect of the smaller amount of dissolved gases except that those species of *Spirogyra*, which grow in running (well-aerated) water, practically never show sexual reproduction. The preceding facts give us an insight into the causes leading to reproduction in the vernal phase; these are the increased intensity of the light, the small percentage of dissolved salts in the water, the rising temperature, and (?) the decrease in amount of dissolved gases. Light is probably the most important, but its effect will be qualified by the increasing concentration of the water as the vernal period passes on; and in the case of a dull spring (without much rainfall), the water may have attained such a degree of concentration by the time the light-intensity becomes adequate, that the latter is no longer able to stimulate the Alga in the direction of reproduction. In fact everything probably depends on each factor acquiring the proper degree of development at the proper time, and in co-ordination with the other influencing factors. It seems very likely that F. F. Blackman's doctrine of limiting factors[1] can be applied to algal reproduction as to other physiological phenomena, and that in the event of the non-reproduction of a species of *Spirogyra* in the spring, one or more of the complex of influencing factors is limiting the process. In the case of a species like *S. varians* (Hass.), Kütz., the combination of factors necessary for reproduction is usually realized in nature, but in other species the reverse is the case (cf. *S. rivularis*, Rabh., in our table; see also Petit, loc. cit., p. 27 (*S. fluviatilis*, Hilse), and Klebs, loc. cit., p. 239).

Our data, as will be seen by a glance at the table, give overwhelming evidence of the reproduction of *Spirogyra* in the vernal phase, and we are only able to mention one example to the contrary. From Comère's and Petit's observations (quoted on p. 425) we gather that matters are very much the same in the districts they investigated. Klebs, however (loc. cit., p. 244), gives a rather different account; he says: 'In der freien Natur pflegen viele Spirogyren im Früjahr und Frühsommer zu kopulieren, doch ohne irgend welche bestimmte Regel; man findet Zygotenbildung ebenso im Sommer, bisweilen sogar im Frühherbst bei sehr sonnigem Wetter.' This statement is not directly contrary to what we have found, but we think it unlikely that reproduction of *Spirogyra* in Germany is not just as predominant in the vernal phase as it is with us; Klebs's own observations, in fact (quoted on p. 428), point in that direction. The phrase 'ohne irgend welche bestimmte Regel' is perhaps therefore not quite exact. That zygospore-formation does go on occasionally in the height of summer and

[1] F. F. Blackman, Optima and Limiting Factors. Ann. of Bot., vol. xix, No. lxxiv, April, 1905, pp. 281–295.

autumn or even in midwinter,[1] is undoubted, and it is quite possible that in some regions owing to certain climatic combinations such phenomena are more abundant than we have found them to be. If reproduction in *Spirogyra* is a result of intensification of certain factors, it is comprehensible that such intensification may occasionally occur at other times of the year than in spring, and that we may consequently find a species reproducing in the autumn or winter.

We may briefly consider the one exceptional case we have observed; this was in a pond at Telscombe, near Newhaven, in which *S. cataeniformis* (Hass.), Kütz., and *S. affinis* (Hass.), Petit,[2] were found reproducing abundantly from September to November, 1904. The following data as to the nature of the weather at this time of the year are taken from Brighton,[3] which is sufficiently near to Telscombe to justify the assumption that there were no marked differences. August and September, 1904, had a good deal more bright sunshine than is their wont (August had 249·26 hours, while the average for the last twenty-seven years was 205·66 hours; September had 192·50 hours, the average for the last twenty-seven years being 163·35). The mean temperature for September, 1904, was 58·1° F., which is about the average (58·4° F.); the extreme temperatures for this month were, however, much less than the average, the range being 44·1° F. to 70·6° F. (as against 35·9° F. to 83·2° F. for the last twenty-seven years). The rainfall was as follows:—July, 1904 = 0·52 in. (average 2·33 in.); August, 1904 = 1·71 in. (average 2·44 in.); September, 1904 = 1·64 in. (average 2·39 in.); October, 1904 = 2·47 in. (average 3·87 in.). The rainfall was therefore considerably below the average. August and September, 1904, were certainly abnormal months meteorologically, and hence possibly the abnormal reproduction of the two species of *Spirogyra*. The very considerably larger number of hours of bright sunshine was probably one of the most important factors.

D. Points of Systematic Interest.

The determination of species of *Spirogyra*, even in the reproductive condition, is combined with so many difficulties that we have thought it best to figure a number of the species examined, so as to leave a permanent record. The following systematic details may also be of some interest.

S. ternata, Ripart, is now usually regarded as a variety of *S. neglecta* (Hass.), Kütz.,[4] and our observations certainly support this view. In a pond in Sydenham Wells Park (July 2, 1906) filaments answering to Petit's

[1] Cf. W. and G. S. West, loc. cit., p. 33.

[2] Cf. Fritsch, Problems in Aquatic Biology, &c., loc. cit., pp. 164-7. The two species are here referred to as '*S. gracilis*.' Renewed investigation of the sample has shown that this determination was incorrect.

[3] Cf. Brighton and Hove Nat. Hist. and Phil. Soc. Annual Report for 1905.

[4] Cf. also Petit, loc. cit., p. 27.

description (loc. cit.) were found. In some filaments zygospores were present in every cell, while in others cells containing zygospores alternated with vegetative cells (cf. Fig. 10); in the latter case the zygospores had their long axes at right angles to the direction of the filament, and the cells containing them were abbreviated and deformed. The vegetative cells were slightly inflated on both sides (Fig. 10) or only on one side (Fig. 11), and had prominent pyrenoids in their chloroplasts. Other filaments of *Spirogyra*, present in the same sample (July 2, 1906) undoubtedly belonged to *S. neglecta*; they were also in the reproductive

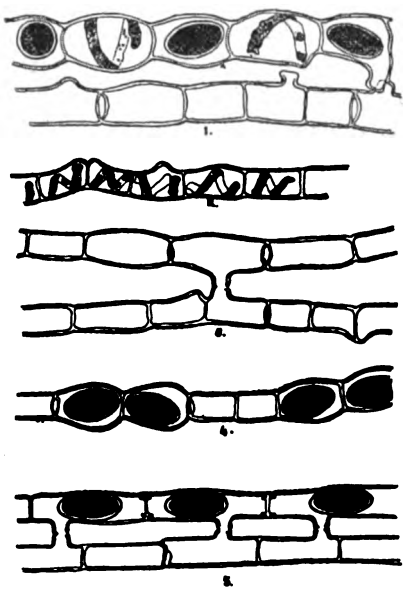

FIG. 1. *Spirogyra varians*. From Tiltham's Pond.
,, 2, 3, 4. *S. affinis*. From Abbot's Pool.
,, 5. *S. Weberi* (Abbot's), magnified 240 times.

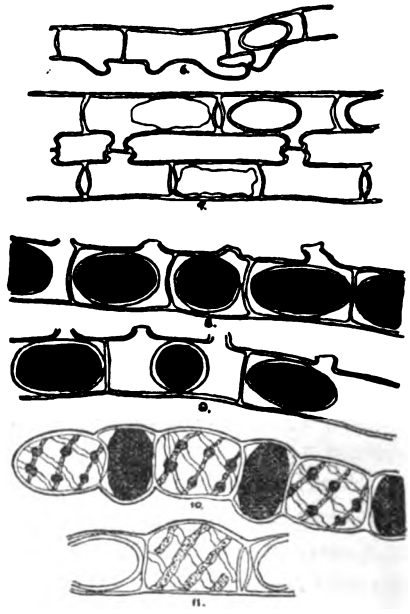

FIG. 6. *Spirogyra longata* from Epsom.
,, 7. ,, ,, from Hendon.
,, 8, 9. *S. neglecta forma* from Abbot's Pool.
,, 10, 11. *S. ternata* from Sydenham Wells.

condition. Earlier samples from the same pond contained abundant *S. neglecta*, but it was almost impossible to identify any of the *Spirogyra* as *S. ternata*.

S. neglecta (Hass.), Kütz., can thus exhibit considerable variation, and we met with another example of this in Abbot's Pool. We were much puzzled in examining samples from this pond to find two *Spirogyras* of the same width, one of which was undoubtedly *S. neglecta*, while the other answered to no description we could find. It differed from *S. neglecta* in having rather broader chloroplasts with larger, better-marked pyrenoids, and in the absence of what Petit calls a 'nervure centrale.' Figs. 8 and 9

give some idea of the different appearance of the zygospores according to their position in the cell; in some cases the ends of the spores were quite pointed, at other times blunt and almost square. For a time we were inclined to regard this form as a distinct species, but we found so many transitions between it and the typical *S. neglecta* that we have come to the conclusion that it cannot be separated from that species; it may possibly prove to be a definite variety.

We may add a few notes regarding other species. *S. varians* (Hass.), Kütz. (Fig. 1), is mentioned by G. S. West[1] as frequently exhibiting lateral conjugation. Although, as will be seen by a glance at the table, we have often found this species in the reproductive condition, we have noticed only scalariform conjugation. In a pond at Hendon, near London (sample collected on April 25, 1905), a long filament of *S. varians* was observed, which was doubled back and conjugating with itself. In Fig. 3 *S. affinis* is shown with both scalariform and lateral conjugation in the same filament. Figs. 6 and 7 show the two types of conjugation in *S. longata.*

E. Summary.

The species of *Spirogyra*, which we have examined, are either purely vernal or exhibit both a vernal and an autumnal phase with an intervening period of scarcity or complete disappearance; it seems possible that there is also a period of disappearance in midwinter, but this is not certainly established. The autumnal reappearance of certain species of *Spirogyra* is no doubt due to the influence of certain combinations of external factors causing a small number of zygospores to germinate; in the absence of these conditions there may be no autumnal phase. Abnormal meteorological conditions may bring about abnormal absence or occurrence of *Spirogyra.*

Reproduction takes place ordinarily in the vernal phase, and is most probably the result of certain periodically recurring combinations of factors, which vary for different species. A considerable number of data are advanced in support of this view. The nature of the stimulus causing vernal reproduction is probably an intensification of those conditions, which are liable to change in spring. Such intensification may exceptionally take place at other times of the year and lead to reproduction at other times than in spring.

[1] A treatise on the British Freshwater Algae. Cambridge, 1904, p. 125.

Spirogyra	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
affinis (Hass.), Petit	'04 I	R, I	I	R	—	—	—	—	**I!**	**I**	**I**	I, R
	'05 A	A	A	A	A	A	A	—	A	A	A	A
	'06 A	A	A	**A**	**A**	**A**	**A**	—	—	—	—	**A**
	'07 A	A	A	A	..	..	..	..	..	..	..	..
bellis (Hass.), Cleve	'04 ..	..	..	R	R	**R**	..	..	..	..	..	..
	'07 ..	..	..	**S**	..	..	..	..	..	..	..	..
cataeniformis (Hass.), Kütz.	'04 ..	..	..	..	..	..	..	..	**I!**	**I**	**I**	I
	'06 ..	..	B	B	**B**	B	—	—	—	—	—	—
	'07 —	—	—	—	B	..	..	..	..	..	..	..
condensata (Vauch.), Kütz.	'04 —	—	—	I	**I**	**I**	—	—	—	—	—	—
Hassallii (Jenn.), Petit	'05 ..	..	..	H	..	..	..	..	..	..	..	..
	'06 ..	..	..	—	—	B	—	—	—	—	—	—
	'07 —	—	—	—	**B**	..	..	..	..	..	..	..
jugalis (Dillw.), Kütz.	'05 A	A	A	**A!**	A, **O**	—	—	A	A	A	A	—
	'06 —	A	A	**T!** A	A	A	A	—	—	—	—	—
	'07 —	—	—	—	..	..	..	..	..	..	..	..
longata (Vauch.), Kütz.	'02 ..	..	..	..	..	**K**	..	..	..	..	..	..
	'05 ..	..	..	**H**	..	..	..	..	..	..	..	..
	'06 ..	..	..	**E**	..	..	..	..	..	..	..	..
neglecta (Hass.), Kütz.	'05 —	—	—	A	**A**	**A**	**A**	**A**	A	—	—	—
	'06 —	—	—	**A**	**A**	**A**	**A**	—	—	—	—	—
	'07 —	—	—	—	..	..	..	..	..	..	..	..
neglecta (Hass.), Kütz., var. *ternata*, Ripart.	'05 ..	..	..	..	..	..	..	..	..	..	..	..
	'06 ..	..	T, W	**T**, W	**T**, W	T, W	**T, W**	—	—	—	—	—
nitida (Dillw.), Link	'02 ..	..	..	..	..	**K**	..	..	..	..	..	..
	'05 A	A	A	A	**A**	A	—	—	—	—	—	—
	'06 ..	..	..	A	A, R	A, R	A, R	R	—	—	—	—
	'07 —	—	—	—	..	..	..	..	..	..	..	..
quadrata (Hass.), Petit	'06 —	—	T	**B, T**	**B**	**B**	—	—	—	—	—	—
	'07 —	—	—	—	B	..	..	..	..	..	..	..
rivularis, Rabh.?	'04 —	—	—	—	—	A	—	—	A	A	A	A
	'05 A	A	A	A	A	A	A	—	A	A	—	—
	'06 A	A	A	A	—	—	—	—	—	—	A	A
	'07 A	A	A	A	A	..	..	..	..	..	..	..
tenuissima (Hass.), Kütz.	'06 ..	..	..	**B**	**B**	**B**	—	—	—	—	—	—
	'07 —	—	—	—	**B**	..	..	..	..	..	..	..
varians (Hass.), Kütz.	'05 —	—	A, H	**A, H**	**A, O**	—	—	—	—	—	—	—
	'06 —	—	A	**A, T, E**	—	—	—	—	—	—	—	—
	'07 —	—	—	**Q, A**	**B, A**	..	..	..	..	..	..	..
Weberi, Kütz.	'02 ..	..	..	..	..	**K**	..	..	..	..	N	—
	'03 —	—	N	N	N	G	..	..	..	..	..	..
	'04 ..	..	..	N	..	..	..	..	..	..	..	..
	'05 A	A	A	A	A, **O**	A	—	—	A	—	—	—
	'06 A	A	A, W?	**A**, W?	**C, A**, W?	A	A	—	—	—	—	—
	'07 A	A	A	A, **S**	..	..	..	..	..	..	..	..

Ordinary type indicates sterile, Clarendon type reproductive condition; where an exclamation mark is added, conjugation only was observed and no zygospores were seen. Wherever a species of *Sp.* is recorded in a pond from which a consecutive series was available its absence is indicated in other months by means of a dash. The letters refer to the different ponds, which are as follows:—

A = Abbot's Pool (series).
B = Barton's Pond (series).
C = Coalville.
E = Chessington.
G = Reigate
H = Hendon.
K = Kew (cf. Fritsch, in Kew Bulletin, Addit. Ser. v, 1906, p. 187, et seq.).
I = Telscombe Pond (series).
N = Penn Ponds (series).
O = Portishead Pond (series).
Q = Pond at Erquy (Brittany).
R = Sheen Common Pond.
S = Pond at Silverdale.
T = Tiltham's Pond (series).
W = Sydenham Wells Pond (series).

NOTE.

ON THE DISTRIBUTION OF CHLOROPHYLL IN THE YOUNG SHOOTS OF WOODY PLANTS.—In the young shoots of many trees and shrubs, the chlorophyll appears to be present not only in the cortex, but also in the cells of the medullary rays and in some of the cells of the medulla. This is particularly well seen in *Jasminum nudiflorum*, Lindl., which flowers in February and March, before the leaves appear. As might be expected, the axis of the inflorescence in this plant possesses the characteristic features of a typical assimilating axis. Xerophily is well marked by the deeply sunk stomata and the much cutinized epidermis. The surface is increased by small lateral extensions of

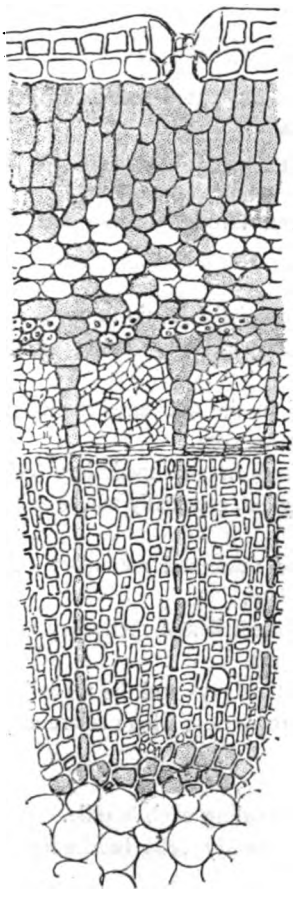

FIG. 1. Transverse section through inflorescence axis of *Jasminum nudiflorum*, showing distribution of chlorophyll.

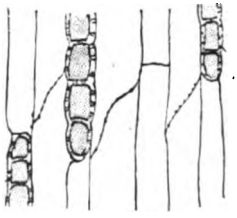

FIG. 2. Tangential section through phloem, showing chlorophyll in medullary rays.

two opposite sides of the square axis; the extended sides alternating in consecutive internodes. The palisade tissue is developed beneath a single colourless hypodermal layer.

The chlorophyll is found not only in the cells of the palisade layer, but in many or all of the cortical cells, in the pericyclic parenchyma bordering on the sieve tubes, in the medullary rays running through the phloem and xylem, and even in some of the medullary cells. The medullary cells that contain chlorophyll border on the protoxylem. They are thicker-walled and slightly smaller than the more internal medullary cells, and are pitted and give the reaction for lignin with acidulated phloroglucin.

Starch was tested for in material that had been picked for several days. It was found in the endodermal layer to the outside of the pericyclic sclerotic fibres, but in no other tissue. Starch was also tested for in material that had just been picked after half an hour's bright sunshine. In this case abundant starch was found in the palisade and cortical cells, in the pericyclic parenchyma bordering on the sieve tubes, and in the medullary rays. No starch could be detected in the cells of the pith.

The following is a list of the plants examined, with a general account of the distribution of chlorophyll in their young shoots. In all cases, with the exception of the evergreens, the material was cut when the leaf-buds were just bursting.

Plant.	Chlorophyll developed in
Tilia europaea, Linn.	Cortex, medullary rays, and the pitted medullary cells bordering on the protoxylem.
Aesculus Hippocastanum, Linn.	Cortex, scantily in the medullary rays, and in the medullary cells bordering on the protoxylem.
Betula alba, Linn.	Cortex, medullary rays, and throughout the medulla which is of small size.
Carpinus Betulus, Linn.	As in *Betula alba*.
Acer Pseudoplatanus, Linn.	Cortex, medullary rays (especially in the very broad ones), and in the medullary cells bordering on the protoxylem.
Ulmus campestris, Linn.	Cortex, medullary rays, and in the thickened medullary cells bordering on the protoxylem.
Populus nigra, Linn.	Cortex, medullary rays, and in the medullary cells bordering on the protoxylem, especially where sclerenchymatous.
Fagus sylvatica, Linn.	Cortex, medullary rays, and thickened medullary cells bordering on the protoxylem.
Fraxinus excelsior, Linn.	Cortex, medullary rays, and thickened medullary cells bordering on the protoxylem.
Quercus Robur, Linn.	Cortex, scantily in the medullary rays and the medullary cells bordering on the protoxylem where sclerenchymatous.
Pyrus amygdaloides, Linn.	As in *Quercus Robur*.
Rosa canina, Linn.	Cortex, and in the cells surrounding each bundle.
Crataegus oxyacantha, Linn.	Cortex, medullary rays, medullary cells bordering on the protoxylem.
Prunus spinosa, Linn.	Cortex, medullary rays, and thickened medullary cells bordering on the protoxylem.
Rubus, sp.	As in *Prunus spinosa*.
Laburnum vulgare, J. Presl.	Cortex, scantily in the medullary rays, and in the medullary cells bordering on the protoxylem.
Robinia Pseudacacia, Linn.	Cortex, medullary rays, scantily in the thickened medullary cells bordering on the protoxylem—particularly clear in the pericyclic parenchyma.

Plant.	Chlorophyll developed in
Sambucus nigra, Linn.	Cortex, scantily in the medullary rays, in a few medullary cells bordering on the protoxylem.
Syringa vulgaris, Linn.	Cortex and lateral extensions of the cortex, medullary rays, and medulla except the very centre.
Corylus Avellana, Linn.	Cortex, medullary rays, cells of medulla bordering on the protoxylem.
Viburnum Lantana, Linn.	Cortex—medullary rays, cells of medulla bordering on the protoxylem.
Lonicera Periclymenum, Linn.	Cortex, medullary rays, cells of medulla bordering on the protoxylem.
Hedera Helix, Linn.	Cortex, medullary rays, and at the points where the rays run into the medulla.
Aucuba japonica, Thunb.	Cortex especially thickened hypodermal layers, scantily in medullary rays and cells of medulla bordering on the protoxylem.
Daphne Mezereum, Linn.	Cortex, medullary rays, medulla.
Cestrum, sp.	Cortex, medullary rays, medulla.
Ilex Aquifolium, Linn.	Cortex only.
Plumbago, sp.	Patches between sclerenchyma in the cortex only.
Larix europaea, DC.	Scantily in outer layers of the cortex only.

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The Cereal Rusts.

I. The Development of their Uredo mycelia.

BY

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With Plates XL–XLIII.

INTRODUCTION.

IN recent years very considerable attention has been attracted to a group of species of *Puccinia*, the more important members of which are parasites of our chief cereals. All these species were at one time included under the three names *P. graminis*, Pers., *P. rubigo-vera*, DC., and *P. coronata*, Corda.

Recently they have been subdivided into thirteen species, each of which is readily recognized by well-defined morphological and physiological characters. These have further been subdivided into no less than twenty-four forms, distinguishable from each other by apparently physiological characters, that of adaptation to particular hosts.

Further, the well-known Swedish mycologist Eriksson in endeavouring to account for special cases of infection was led to formulate a view since known as the 'Mycoplasm' hypothesis, according to which the mycelium perennates in the form of a naked intercellular plasma.

Unfortunately our knowledge of the cytology of these fungi, notwithstanding the interesting work which had been done by De Bary (4), Poirault and Raciborski, Sapin-Trouffy (31), Marshall Ward (33) and Blackman (6), is still in a very unsatisfactory condition.

It was obviously desirable that someone should undertake the detailed investigation of the histology of these forms.

It was under such circumstances that the late Professor Marshall Ward very kindly suggested that I should attack, partly in conjunction with himself, but mainly working alone under his advice, some of these problems.

The work was begun in November, 1903, and was carried on for two years in the Cambridge Botany School, and has since been continued in my own Laboratory at Pretoria.

As would be expected, a vast amount of information relating to the

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histology, physiology, and ecology of these Fungi has now been obtained, in addition to many interesting facts connected with the relative immunity of the various hosts.

The development of the Uredo mycelium of the Fungi (to be described) has been followed in detail from the time of spore-germination to spore-production. In such a course of development we have to recognize three distinct stages:—

1. The attack by the parasite on its host, or the first phenomenon of occupation.

2. The course taken after occupation by the further growth of the parasite.

3. The reaction on the host after occupation and the subsequent reciprocal action of host and parasite.

It is mainly to the attack by the parasite on its host, or the first phenomenon of occupation, that we shall confine our attention in this paper.

Up to the present the complete series of these rusts has not been obtained, but as I have examined all the species which are known to occur on Cereals, it is thought advisable to publish them without further delay, and leave several species which occur on the more uncommon grasses for a future investigation, should they be obtained.

A detailed histological study has also been made of the so-called 'physiological species,' 'biologic species,' 'sister species,' or 'specialized forms' of rusts, and points of great interest have come to light. These I hope to publish at an early date.

Methods.

The methods adopted for studying the histology are as follows:—

1. Selection of host plants. For infection purposes, seedlings of plants known to be susceptible to the particular kind of rust which it was desired to study were raised. In most cases, directly the first foliage leaf was fully expanded, a series of inoculations were made by laying the spores on the inner face of the leaves with a flat platinum needle. Sowings of spores were also made at the same time in watch glasses of distilled water as control experiments to observe the germination capacity of the spores.

2. After inoculation damp bell-jars were placed over the plants for forty-eight hours.

3. The inoculated portions of the leaves were then removed and fixed daily until the crop of uredo-pustules made their appearance. As a rule this generally took place about the tenth day, but was liable to considerable variation, according to the different rusts under investigation.

In this way the life-history of the Fungus was studied from the period of germination of the spore up to the time that it had successfully reproduced itself on its particular host.

THE FIXING SOLUTIONS.

1. Chrom-acetic solutions. On the whole the best results were obtained with this solution, in strengths varying from 1 per cent. to one-tenth per cent. solutions. Whenever possible the fixing was done under the air-pump, and the fixed portions were allowed to stay in the solution from twelve to forty-eight hours.

2. Flemming's Chrom-osmium acetic solution. The weaker formula was most frequently used, and sometimes it was diluted down to one-half its volume with water. The chief objections to these solutions are:—

(*a*) The blackening due to the osmium.

(*b*) Their slow penetrating power.

(*c*) They cause the protoplasm of the hyphae to become vacuolated.

3. Picro-formol solution:—

Commercial formol . . .	30 c.c.
Water	20 c.c.
Acetic acid	5 c.c.

saturated with picric acid.

The advantages of this solution over the two previous ones are:—

(*a*) Its quick penetrating power.

(*b*) Its not causing the material to become brittle on cutting.

After washing and hardening in the usual way, the material was taken into paraffin by means of Xylol or cedar-wood oil. The sections were cut from 5 to 10 μ thick, and all the staining done on the slide.

The chief stains used were:—

1. Diamant Fuchsin and Lichtgrün.
2. Safranin, gentian-violet and orange G.
3. Iron-alum-haematoxylin with safranin, eosin or orange G.

GENERAL.

The Uredineae, like most other true parasites, possess two methods of penetrating their host.

In the one case the germ-tube emitted from the spore penetrates the firm membrane or cuticle. This usually takes place with the sporidia developed from the teleutospores.

In the other case, the germ-tubes penetrate their host by means of a natural opening or orifice, the stoma. This is always the method adopted in the case of uredospores and aecidiospores.

The method of infection by means of uredospores was known to de Bary, who has given a general description of the sequence of events involved. Several other investigators have from time to time given various descriptions of infection phenomena. But no one seems to have realized

that each set of phenomena is of a very definite nature for each species of Uredo, i. e. the infection phenomena presented by *Uredo graminis* on wheat is always of the same definite type and differs markedly from say *Uredo triticina* on wheat or any of the other Uredo forms to be described, which again in their turn are all constant. In uredospore infection it is usual to recognize two distinct steps: entry or inoculation and true infection.

In addition to the preliminary act of germination, entry or inoculation includes the swelling up of the tip of the germ-tube over the stoma, to form the appressorium. From the appressorium a slender branch passes through the stomatal slit and at once swells out into a vesicular body, the substomatal vesicle, into which the whole contents of the spore is poured. The entry of the fungus is now assured.

The next step in the development is the putting forth from the substomatal vesicle of one or more infecting hyphae, which closely apply themselves to the host-cells and send in their haustoria, thus bringing about the act of infection.

Puccinia graminis Tritici, Erikss. and Henn.

The Black Rust of Cereals, which is heteroecious on Barberry, has been broken up by Eriksson (12) into six specialized forms:—

1. *Puccinia graminis Secalis*, found on *Secale cereale*, *Hordeum vulgare*, *Agropyrum repens*, *Elymus arenarius*, and *Hordeum comosum*, but not on *Triticum vulgare* or *Avena sativa*.

2. *Puccinia graminis Tritici* on *Triticum vulgare*. He regards this form as 'not sharply fixed', because he says Uredo infection from Wheat feebly infects Barley, Rye very slightly, and in the case of Oats he regards the infection as doubtful.

3. *Puccinia graminis Avenae* on *Avena sativa*, *Alopecurus pratensis*. *Dactylis glomerata*, but not on *Triticum vulgare*, *Hordeum vulgare*, or *Secale cereale*.

4. *Puccinia graminis Airae* on *Aira caespitosa* only.

5. *Puccinia graminis Agrostis* on *Agrostis canina* and *A. stolonifera* only.

6. *Puccinia graminis Poae* on Poa only.

In this paper we shall concern ourselves solely with the form *Uredo graminis Tritici*, Erikss. and Henn., found on Wheat.

This rust, formerly so common in England, now appears to have been replaced by the Yellow Rust of Wheat, *Uredo glumarum*, which is particularly abundant in the Fen District.

Uredo graminis is the rust responsible for the damage wrought in the Cereal crops of the United States of America, Australia, and Tasmania.

In India, according to Barclay (3), it is comparatively unimportant.

In South Africa, especially the Transvaal, *Uredo graminis* prevents the successful raising of summer cereal crops.

Uredo graminis, the Black Rust, is so called from the dark colour produced on the straw by the teleutospore patches. In the field the Uredo stage is recognized by its rusty orange to brown ochre coloured pustules, which are from 12–15 mm. long or even longer. Under the microscope the spores are broadly elliptic, dirty yellow, spinulose, 17–40 × 14–22 μ.

The Histology of Uredo graminis.

Although *Uredo graminis* has probably received more attention at the hands of mycologists all the world over than any other parasitic Fungus, I think it will be clear that the complete sequence of infection as represented in my figures has never heretofore been observed or represented. Apart from de Bary (5), Marshall Ward (32), and more recently Bolley (7), have figured the germination of and infection by means of uredospores of *Uredo graminis*.

De Bary has figured a slight swelling of the germ-tube over the stoma, but nothing more. Bolley has depicted the germ-tube passing straight through the stoma to the mesophyll-cells below. This I find never occurs.

Further, Bolley (7), p. 630, says that the germ-tube from these uredospores 'may bore its way through the skin of a wheat plant and thus start another point of infection'; in the case of uredospores of '*Puccinia rubigo-vera*,' he illustrates some of the filaments passing directly through the leaf. I have now examined some hundreds of thousands of uredospore infections on all the cereal crops and various other Gramineae, and have never met with a single infection which was not brought about by way of a stoma.

The germination of the uredospore on the epidermis is usually effected within the first twenty-four hours, and by the third day infection is well established.

When the spore germinates, two germ-tubes frequently appear at first, but one quickly gets ahead of the other and arrests its growth. The tip of the germ-tube, as soon as it reaches a stoma, instead of entering straight away, swells up and forms the appressorium (Figs. 1 and 2). From the appressorium a narrow branch passes through the stomatal slit, always situated somewhat nearer to one of the corners. As soon as the neck has got through the aperture, it enlarges to form the sub-stomatal vesicle, which applies itself closely to the inner face of the stoma (Figs. 2 and 3). Into this vesicle the whole contents of the spore are poured, and the entry of the fungus is completed. The germ-tube and appressorium now quickly wither and are soon lost sight of.

A point to be noted in connexion with the substomatal vesicle, both in

these species and those to be described below, is that it always occupies a definite position relative to the stoma, i.e. in *Uredo graminis* and most other species its long axis is always parallel to the stomatal slit. From one end of the substomatal vesicle the infecting hypha is given off, which at once penetrates the host cells by means of its haustoria (Fig. 3), and thus a successful infection of the host is accomplished.

A very characteristic feature connected with the substomatal vesicle of this species is that the end from which the infecting hypha springs always clings very closely to the head of the guard cell, or the epidermal cell abutting on it.

From the position of the organs depicted in Figs. 2 and 3 it will be clear that the complete sequence of infection, as has usually been figured, cannot be attained by transverse section; for the relative position of these organs is such as to make this almost impossible with anything like moderately thin sections. In Fig. 4 is shown such a transverse section, which happens to pass through the point of union of appressorium and substomatal vesicle.

In diameter the appressorium is 9 μ, while in length it is roughly 27 μ, the substomatal vesicle being approximately of the same dimensions. The hyphae are of uniform size, and measure 3·5 μ in thickness.

The haustoria are very varied in shape. They attain their greatest development in the cells bordering on the vascular bundles, where they often become very branched and contorted. A very young haustorium, which has just penetrated one of the mesophyll cells, is seen in Fig. 5, while in Fig. 6 forms commonly found in the epidermal cells are shown.

Puccinia Phlei-pratensis, Erikss. and Henn.

The Timothy rust was set apart from *P. graminis* in 1894 by Eriksson and Henning (16) as a distinct species, because out of ninety-two infections with good germinating material on *Berberis*, only one feeble infection resulted. From this they concluded that this form does not probably form its aecidium on *Berberis*.

Further experiments on *Berberis* by Eriksson (10) (14) have simply served to confirm this result. He has also shown that it can be transferred in the Uredo stage from *Phleum pratense* to *Festuca elatior* and vice versa (Eriksson) (9); and in addition that it will feebly infect *Phleum Michelii*, *Secale cereale*, and *Avena sativa* (Eriksson) (14), but does not infect *Triticum vulgare*, *Hordeum vulgare*, and *Poa pratensis*.

Consequently Eriksson regards *P. Phlei-pratensis* as 'nicht scharf fixiert'.

P. Phlei-pratensis is distinguished in the Uredo stage by the dark yellowish-brown pustules which are from 1–2 mm. long; these may frequently run together in a long line as much as 10 mm. long or more on

the leaf edges and sheaths. The spores are oblong, somewhat pear-shaped, spinous, and of a dirty yellow colour. They measure from 18–27 × 15–19 μ. It is interesting to note that according to Eriksson and Henning (16) the Uredo mycelium is able to survive the winter. For during 1891–2 fresh pustules were produced on the 28th of December, and they continued even up to as late as the 28th of March, and during the winter 1892–3 the latest and first appearance of fresh pustules was on the 30th of November and the 27th of March.

Also, according to them, the germinating capacity of the uredospores falls with the increase in severity of the winter.

Germination of the Spore.

From the spore on germination a fine delicate germ-tube runs along the surface of the epidermis, and when the tip reaches a stoma, it swells up a little into a thin and delicate vesicle, the appressorium. Directly the contents have passed from the appressorium into the sub-stomatal vesicle, the delicate and now empty appressorium shrivels up and breaks away at its junction with the firmer walled sub-stomatal vesicle, and so is rarely seen after the formation of the latter.

The sub-stomatal vesicle is a very definitely shaped body (Fig. 7), closely resembling that of *Uredo graminis*, but narrower. Like that of *Uredo graminis*, it gives rise at one end to one infecting hypha only, from which the others subsequently arise. In diameter it measures from 4–6 μ, and in length from 30–35 μ. It differs from *Uredo graminis* chiefly in the fact that the end from which the hypha springs does not cling to the head of the guard-cell.

The resulting hyphae, and especially their haustoria, bear a very striking resemblance to those of *P. Symphyti-Bromorum*. As a general rule the hyphae are thinner and more thread-like than even those of *P. Symphyti-Bromorum* or *P. simplex*.

The first formation of septa in the hyphae occurs about the third day, and always takes place in connexion with the development of haustoria.

In addition to these septa, others appear at longer or shorter intervals in the branched hyphae up to about the tenth day, when, with the preparation for spore production, the hyphae become drained of their protoplasm and uniform septation occurs in all the older hyphae. So that at this stage the only hyphae which are full of contents, and for the most part are unseptate, are those which run in the margins of the pustules, the so-called 'Protomycelium.'

Nuclei.

The nuclei closely resemble those of *P. Symphyti-Bromorum*, *simplex*, and *coronifera*, and are usually of an oval shape, and in the vegetative hyphae they all divide by the direct method of division. Two such nuclei are shown in Fig. 12.

Haustoria.

The haustoria of this form, both in the earliest stages of development and when they reach maturity, bear very striking resemblances to those of *P. Symphyti-Bromorum* as figured by Marshall Ward (33). This is especially noteworthy, because Eriksson ('04) (15) in his last paper on 'The vegetative life of the Cereal Rust Fungi' expressly states that 'In no single case has he found such young stages of haustoria as those figured by Marshall Ward' (33).

Consequently I have thought it advisable to show that such stages of young haustoria are to be found in this form (*P. Phlei-pratensis*). See Figs. 8, 9, and 10.

Haustoria are produced from the apices of the hyphae.

Before a haustorium is formed the hypha becomes distinctly swollen to a distance of about 10 μ from the extreme tip. This swollen portion becomes cut off by a transverse wall, and contains a protoplasm which is more vacuolar than that of the rest of the hyphae.

In it two or more nuclei of much smaller size than those in the hyphae are seen.

Simultaneously with the transverse wall which cuts off the haustorium, a tube-like structure begins to grow out and pierce the host cell-wall. After penetrating the wall, the little tube then begins to swell at its distal end, and very soon a brightly staining granule is seen in its interior, and this generally lies near the aperture of the tube.

As the body of the haustorium increases in size, its proximal swollen end becomes quite empty, and the small round nuclei which were present in it are now seen to have passed over into the distal end of the haustorium (Fig. 11). The large haustorium embracing the host cells nucleus in Fig. 11 is the form commonly seen in the chlorophyll containing cells, while the small hammer-headed forms are rather common in the epidermis.

Puccinia Glumarum (Schmidt), Erikss. and Henn.

This is the Yellow rust of wheat, by far the most prevalent of rusts on the wheat fields around Cambridge. In South Africa as yet I have not found this rust, and doubt whether it exists here.

It was originally included under *P. rubigo-vera*. DC. (*P. striaeformis*, Westd., and *P. straminis*, Fuck.).

It was first distinguished as a single species, with very distinct morphological and biological characteristics by Eriksson and Henning (16), and a few years later Eriksson divided the old *P. rubigo-vera*, DC., into seven additional forms, so that to-day under *P. rubigo-vera*, DC., he includes:—

1. *P. glumarum* (Schm.), Erikss. and Henn.
   The Yellow rust. Aecidium unknown.
2. *P. dispersa*, Erikss.
   The Brown rust of the rye. Aecidium on *Anchusae*.
3. *P. triticina*, Erikss.
   The Brown rust of wheat. Aecidium unknown.
4. *Symphyti-Bromorum*. Müll.
   The Brown rust of Bromes. Aecidium on *Symphytum*.
5. *P. agropyrina*, Erikss.
   On *Triticum repens*. Aecidium unknown.
6. *P. holcina*, Erikss.
   On *Holcus lanatus*, *H. mollis*. Aecidium unknown.
7. *P. Triseti*, Erikss.
   On *Trisetum flavescens*. Aecidium unknown.
8. *P. simplex* (Kcke.), Erikss. and Henn.
   The dwarf rust on *Hordeum vulgare*.

Eriksson (9) divides *P. glumarum* into five specialized forms.

1. *P. glumarum Tritici*, Erikss.

In which uredospores from wheat will only infect wheat, but not barley or rye.

2. *P. glumarum Hordei*, Erikss.

Uredospores from barley will only infect barley, not rye or wheat.

3. *P. glumarum Secalis*, Erikss.

Uredospores from rye infect only rye, not barley or wheat, although Eriksson says he once got a feeble success from rye to wheat, and therefore is inclined to regard his form as 'nicht scharf fixiert'. But we need further experiments on this point before it can be regarded as such.

4. *P. glumarum Elymi*, Erikss.

Uredospores from *Elymus arenarius*, L., will not infect either wheat barley, or rye.

5. *P. glumarum Agropyri*, Erikss.

Uredospores from *Agropyrum repens*. Beauv., will not infect rye, wheat, or barley.

P. glumarum is also found on the following Bromes:—*Bromus adoensis*, *asper*, *arvensis mollis*, *secalinus*, *sterilis*, *tectorum*.

The Uredo stage of *P. glumarum* is readily distinguished in the field by its bright yellow colour, sulphur to pale cadmium. The pustules have a great tendency to run in long rows up and down the leaf, sometimes producing a disease spot as much as 70 mm. long, but this is by no means

always the case, for frequently the Fungus will invade a leaf from an infected area in the most methodical fashion, e. g. it will spread uniformly through the leaf blade both up and down the leaf from an infected area at the rate of 10–12 mm. per day, that is to say the total length of leaf infected per day usually amounts to 20–24 mm.

The pustules are always much more numerous on the inner face of the leaves than the outer.

They are from ·5–1 mm. long and ·3–·4 mm. broad.

The spores are round or shortly elliptical echinulate and yellow, measuring 25–30 μ in diameter.

The uredospores are easily distinguished under the microscope through their colourless membrane from the spores of the Brown rust with its brownish coloured membrane.

An aecidium of *P. glumarum* has so far not yet been discovered, and this is not by any means surprising, for very few trials appear to have been made in this direction.

Eriksson, it is true, has tried to infect with the teleutospores (which like those of *P. dispersa* germinate directly they are ripe) *Anchusa arvensis*, *officinalis*, *vulgaris*, *Cynoglossum officinale*, and *Pulmonaria officinalis*, but with no success.

Around Cambridge the Uredo pustules are to be found all the year round, both on the side tillers in the unploughed stubble fields and also on the fields of young winter corn. It should be mentioned that especially during the months of January and February it is those leaves of the winter corn which lie or come into contact with the damp soil that are very liable to show the uredo pustules and characteristic yellow flecks.

The Histology of Uredo glumarum.

The following works dealing more or less with the histology of *P. glumarum* have already appeared:—

Eriksson and Henning (17) gave a short description of the anatomy of the Yellow Rust with a few figures.

Klebahn (24) in a short article on 'The anatomy of the Yellow Rust' drew attention to the great thickness of the hyphae in this species when compared with those of the other cereal rusts. He also suggested that Eriksson had probably mistaken the haustoria of these rusts for his 'mycoplasm.'

In January, 1904, Eriksson and Tischler (18) published as the first of a series of articles 'Über das vegetative Leben der Getreiderostpilze' a paper on '*Puccinia glumarum* (Schm.), Eriks. and Henn., in der heranwachsenden Weizenplanze'.

This paper deals with the mycoplasm hypothesis. Both normal and abnormal hyphae are figured, and Eriksson maintains that one is only a stage in the development of the other.

In May, 1904, Klebahn (25) in 'Bemerkungen über das Mycel des Gelbrostes und über die neueste Phase der Mycoplasma-Hypothese' tries to find an explanation for some of the phenomena represented in Eriksson and Tischler's paper.

In June, 1904, Eriksson (15) published a second paper on *P. glumarum*, entitled '*Puccinia glumarum* (Schm.), Eriks. and Henn., in der heranwachsenden Gerstenpflanze', the third of the series, 'Über das vegetative Leben der Getreiderostpilze.'

But as this paper is solely concerned with what Eriksson still calls the mycoplasm stage of the Fungus, it does not concern us here, for we shall confine our attention for the present to the normal histology.

In conjunction with Marshall Ward (34) I studied the histology of this rust in very great detail. It has been examined in a number of varieties of wheat, under various conditions of growth, normal as well as abnormal. Two varieties were used rather more extensively than the others for this purpose.

1. The one known as 'Red King', because it was one of the most susceptible of the many varieties grown by Biffen at the University Experimental Farm.

2. The other was Michigan Bronze, the same variety as that with which Eriksson worked. This was chosen partly because it also is a very susceptible wheat to Yellow rust, and partly because it had been specially recommended by Eriksson.

Histologically the most striking point of difference between the Uredo of *P. glumarum* and the other forms under discussion is that in *P. glumarum* the hyphae are extremely thick, frequently measuring from 10–19 μ across, and at an early stage are seen to be crammed with nuclei; whereas in the other forms the hyphae are only 3·5 μ thick, and have but few nuclei arranged usually in single file.

Germination of the Spore.

The spore on germinating puts out a simple and very delicate germ-tube, which runs along the surface of the epidermis until it comes to a stoma, where the tip of the germ tube swells slightly and forms a fragile vesicle, the sub-stomatal vesicle, which applies itself closely to the inner face of the stoma.

Into this large and thick-walled vesicle the whole contents of the spore are poured, and it is frequently seen crammed with nuclei only forty-eight hours after infection (Pl. XL, Figs. 13, 14, and 15).

This sub-stomatal vesicle is generally of a definite shape, being usually cylindrical with rounded ends.

In diameter it measures from 8 to 19 μ. Its position relative to the stoma is also very definite, for it lays itself with its long axis parallel to

the stomatal slit, so that in a transverse section of the stoma it is seen as a circular body blocking up the stoma (Fig. 16), and in longitudinal section as a long oval body fitting against the guard cells.

With the formation of the sub-stomatal vesicle we may regard the Fungus as having effected an entry into its host.

The next step in the development is the formation of the true infecting hypha.

This springs from one end of the sub-stomatal vesicle, and either strikes straight across the sub-stomatal intercellular space or runs between the mesophyll-cells and the epidermis.

The infecting hypha at once forms a haustorium in the first cell with which it establishes contact, and then we may say that infection has taken place. Frequently, in the same section, spore, germ-tube, appressorium, sub-stomatal vesicle, and the first infecting hypha with its haustorium, may be seen.

Before describing the hyphae and their general behaviour, it should be pointed out at once that they always possess a definite membrane which is clearly visible from their first formation to the production of spores.

Even in cases where the hypha fits itself into large and irregular intercellular spaces, the outer membrane is well developed. From the third day onwards to the seventh day the hyphae attain to the maximum development, and two distinct kinds of branches are seen.

1. The short branches, which are frequently septate and ramify in the intercellular spaces between the chlorophyll-containing cells.

2. The long branches or runners. These are very vacuolated, very rarely septate, and apparently do not form haustoria.

These run up and down the leaf, and are the hyphae which cause the rapid spread of the Uredo pustules (Fig. 22).

One of these hyphae running from the margin of a Uredo pustule will in twenty-four hours attain a length of 12 mm., it then branches repeatedly at its apex, forming a nest of hyphae, which in the next twenty-four hours form the beginnings of a new Uredo pustule.

Septation of the Hyphae.

There are three distinct formations of septa:—

1. Those formed in connexion with the haustoria. Directly a haustorium is formed from the tip of a hypha, a transverse septum makes its appearance and cuts off the tip. These septa may be formed as early as the third day.

2. In the same way septa are formed in the short branches of the hyphae, which become so abundant in the intercellular spaces from the sixth day onwards.

3. Uniform septation of the normal hyphae does not occur as a rule until the day before spore formation, so that it is brought about in a comparatively short space of time.

Nuclei.

Each nucleus consists of a central body which stains as a deep homogeneous mass with fuchsin, gentian violet, and haematoxylin.

This central body lies in a clear space, the nuclear zone, and in the young and actively growing hyphae, no definite nuclear membrane can be made out surrounding it. But in the older hyphae, especially in the long runners where the protoplasm is more vacuolated, and the nuclei not quite so numerous, the typical resting nucleus is seen. Here it consists of a central body lying in a clear area surrounded by a definite nuclear membrane, and in some cases connecting strands can be seen running from the central body to the nuclear membrane.

In connexion with the nuclei, which must be in an active state of division in the young hyphae, distinct centrosomes can be made out. These appear as a single, deep-staining small body just outside the border of the nuclear zone. As a rule they are surrounded by a clearer space, but no definite membrane or radiations are to be seen (Fig. 15). The nuclei divide very rapidly up to the sixth and seventh day, when a change begins to take place. Some of them no longer stand out sharply, but they appear as dull red specks with no distinct membrane, and there is a great tendency for these small and degenerating nuclei to mass together in patches so that the hyphae often show a corroded appearance. The surviving nuclei are conspicuous by their large size and staining capacity.

Haustoria.

The commonest form for *P. glumarum* is a small club-shaped body, which is very frequently seen in close connexion with the nucleus of the cell (Fig. 24), whereas the hammer-headed form so frequently met with in *P. Symphyti-Bromorum* is seldom seen.

The haustoria often contain as many as five nuclei, and attain their largest dimensions in the cells surrounding the vascular bundles, where they are usually very numerous and much branched. In no case have they been seen in the vascular elements themselves.

As the haustoria become older, they become very thick walled, and have an empty or very vacuolated appearance, while the wall stains very deeply and the nuclei can no longer be made out. Arborescent forms, such as are shown in Figs. 18 and 19, are frequently found.

Preparation for Spore Formation.

Towards the eighth day the hyphae become very vacuolated, contain fewer nuclei, which are frequently of an oval shape and stain very deeply.

The hyphae from now onwards become extremely thin (Figs. 20 and 21), from 3·5–6 μ in thickness, and always have a great tendency to make for the stomatal cavities. Here they branch very rapidly, forming a dense weft, giving rise to the Uredo sori, which are seen bulging out the cells in the region of the stomata.

Puccinia dispersa, Erikss.

This is the Brown rust of the rye, originally included under the old *P.* '*Rubigo-vera*' (DC.), as *Aecidium asperifolii*, Pers.

Eriksson (9) was the first to point out the characteristics of the 'Braunrost' (*P. dispersa*, Erikss. and Henn.), and very soon afterwards he split it up into the specialized forms (f. sp. *Secalis*, *Tritici*, *Bromi*, *Agropyri*).

Later on Eriksson (11) showed that the brown rust of the rye *P. dispersa*, Erikss. (not Erikss. and Henn.), is a distinct species from the other brown rusts, for its uredo and teleutospores are only found on *Secale cereale*, L., and *S. montanum*, Guss., not on *Triticum*, &c.

The aecidium is found only on *Anchusa arvensis* and *A. officinalis*. The teleutospores are capable of germination immediately after their formation, and as was shown by Eriksson and confirmed by Klebahn (22), when the teleutospores from *Secale cereale* are sown on *Anchusa arvensis* and *A. officinalis* the aecidia are always produced, whereas sowings of the teleutospores from *Triticum vulgare*, *Bromus mollis*, *Bromus macrostachys* failed to produce the aecidia on *Anchusa*. The aecidiospores from *Anchusa arvensis* and *A. officinalis* always successfully infected *Secale cereale*, but these aecidiospores when sown on *Triticum vulgare*, *Bromus arvensis*, *Hordeum vulgare*, *Avena sativa*, *Agropyrum repens*, *Holcus lanatus* produced no results.

Also uredospores from *Secale cereale* would only successfully infect *Secale cereale*, but failed when sown on *Triticum vulgare*, *Bromus mollis*, *Bromus arvensis*, *Agropyrum repens*, *Holcus lanatus*, *Trisetum flavescens*, to produce the Uredo pustule.

In the field the uredo stage is distinguished by the pustules being from 1 to 1·5 mm. long, nearly 1 mm. broad, and scattered irregularly over the leaf. As a rule the pustules are of a Terra Sienna colour (but too much reliance must not be placed on colour).

The spores are usually round, spinous, of a dirty yellow, and measure in diameter 19–20 μ.

THE HISTOLOGY OF UREDO DISPERSA, ERIKSS.

The general development of the mycelium of *P. dispersa* is very similar to that of *P. glumarum* and *P. Symphyti-Bromorum*, and the other species to be described.

Morphologically the mycelium of *P. dispersa* more closely resembles that of *P. Symphyti-Bromorum* than that of *P. glumarum*, but it is at once distinguished from either of these two species by its characteristically shaped sub-stomatal vesicle.

When the spore germinates a delicate germ-tube is formed, which may branch repeatedly until the tip reaches a stoma, where it swells up and forms the appressorium (as is seen in the oblique section of Fig. 25).

A short tube grows out from the appressorium, which passes through the stoma, and then immediately swells out into the large substomal vesicle, which differs very strikingly in shape from that of *P. Symphyti-Bromorum*, which is more triangular and does not become transversely septate.

In diameter the substomatal vesicle is from 11–14 μ, and like that of *P. glumarum*, it lays itself with its long axis parallel to the stomatal slit (see Figs. 26 and 27), but it differs from *P. glumarum* in that it gives off at each end an infecting hypha, and it very soon becomes divided by a transverse septum, which never takes place in the substomatal vesicle of *P. glumarum*.

The Hyphae.

The hyphae in general appearance and structure are very like those of *P. Symphyti-Bromorum*, containing few nuclei, which are usually seen in pairs and arranged in single file in the hyphae. The hyphae measure from 3–5 μ across, and in the young condition are full of protoplasm and non-septate, but as they grow older from the sixth day onwards they lose their protoplasmic contents and become septate, so that at the time of pustule formation the hyphae become quite empty and closely septate, forming a dense pseudo-parenchyma.

Nuclei.

The nuclei in the young and actively growing hyphae are of an oval shape, consisting of a single deeply staining body surrounded by a clear space, the nuclear zone. Within this clear space a distinct centrosome can usually be made out towards the polar end (Fig. 33). No definite nuclear membrane can be made out in connexion with the nuclei in this condition. But in the resting condition the typical nucleus, as has already been described under *P. glumarum*, is seen.

The Haustoria.

The haustoria of *P. dispersa* are much more uniform in shape than those found with *P. glumarum* or *P. Symphyti-Bromorum*.

The common forms met with are those shown in Fig. 34; branch forms are very rarely found.

Puccinia triticina, Erikss.

The Brown rust of wheat, now known as *P. triticina*. Erikss., is probably identical with the rust still known in the United States as the 'orange-leaf rust of wheat,' *P. rubigo-vera Tritici*, originally included under *P. rubigo-vera*, DC. (*P. straminis*, Fuck. = *P. striaeformis*, West). Eriksson and Henning (16) first sorted it out under the name *P. dispersa* forma specialis *Tritici*.

Five years later Eriksson (11) showed that the brown rust of wheat was a distinct species, which he named *P. triticina*, Erikss. For he found that unlike *P. dispersa* the teleutospores germinated only after the winter, they would not infect *Anchusa* through their sporidia, and no infection resulted on wheat with the aecidiospores from *Anchusa*.

In the field *Uredo triticina* is easily distinguished from *Uredo graminis* by its smaller pustules, which usually are found on the leaves only. The pustules measure from ·3–·5 mm. in length and ·1–·2 mm. in breadth, and are closely crowded together on the leaf surface. Large erumpent pustules so characteristic of *Uredo graminis* never occur. The spores are round to shortly elliptical, 19–20 μ × 24–25 μ, smooth and dirty yellow in colour.

The Histology of Uredo Triticina.

When we come to compare the histology of *Uredo triticina* with that of *Uredo dispersa* we at once find a striking difference between the infection organs of these two parasites, as will be seen by comparing Figs. 25–27 with Figs. 28–30.

The substomatal vesicle of *Uredo triticina* is globular in shape, and gives origin to a single infecting hypha, from which other hyphae may eventually arise. A typical vesicle is shown in Fig. 28. In length this vesicle is 21 μ, breadth 12 μ, and diameter 15 μ.

In Fig. 30 is shown a transverse section through a stoma, in which the appressorium, neck, substomatal vesicle, and infecting hypha with the first haustorium is seen.

The resulting hyphae are very similar to the forms already described (with the exception of *P. glumarum*) and need little mention.

Puccinia Symphyti-Bromorum, F. Müll.

Originally the Brown rust of the Bromes was placed together with the Brown rust of Rye under the name *Puccinia rubigo-vera*, DC. Eriksson (9) was the first to separate it off as *Puccinia dispersa*, Erikss. et Henn., forma specialis *Bromi*.

Later on he regarded it as a distinct species, *Puccinia bromina*, Erikss. (11), from the fact that he was unable to infect plants of Rye with uredospores from the Bromes, and vice versa to infect Bromes with uredospores from the Rye.

Müller (26) (27) showed that the Aecidium of this species is formed on *Symphytum officinale*, L. and *Pulmonaria montana*, Lej., and for this reason it has now been called *P. Symphyti-Bromorum* by Klebahn.

Not only is it to be distinguished by these physiological differences from *P. dispersa* on Rye, but as will be seen from my figures, the uredo mycelium of *P. Symphyti-Bromorum* is morphologically distinct from that of *P. dispersa*.

As the histology of *P. Symphyti-Bromorum* has been very fully described by Marshall Ward (33) there is no need to devote any further space to it here, except to say that my observations entirely coincide with those of Marshall Ward.

To avoid confusion in nomenclature it should be noted that this *P. dispersa* examined by Marshall Ward (33) is now known as *P. Symphyti-Bromorum*.

Puccinia simplex (Kcke.), Erikss. and Henn.

This is the brown or dwarf rust of the barley. It is very common on the barley fields around Cambridge, and is to be found there in the uredo stage all the year round. Up to the present I have not found this rust in South Africa.

By the earliest observers it was distinguished under various synonyms, e.g.

(*P. straminis* var. *simplex* Kornicke, 1865; *Uromyces Hordei* Nielsen, 1875; *P. anomala*, Rostrup, 1876).

However, Eriksson and Henning (16) have shown that it must be regarded as an independent species.

In the field it is characterized by its extremely small and very scattered pustules, which in length are from ·3–·5 mm. and in breadth ·1–·2 mm. The uredospores are spherical to oval, with a brownish coloured membrane, which is distinctly spiny. They measure from 20–30 μ by 17–22 μ.

The teleutospores according to Klebahn are mostly one-celled, and germinate only after the winter's rest.

The Aecidium is as yet unknown, Klebahn during 1900 and 1901 made sowings on some forty-three plants, but with no success.

Klebahn (24) has found *P. simplex* on *Hordeum vulgare*, L., *vulgare* '*cornutum*,' *coeleste trifurcatum*, *distichum*, L., *distichum* '*nigricans*,' *hexastichum*, L., *zeocriton*, L.

The Histology of Uredo simplex (Kcke.), Erikss. and Henn.

Klebahn (24) in two figures on the anatomy of this rust, shows the general appearance of the hyphae and some young haustoria. He

says the mycelium shows nothing remarkable and gives the measurement of the hyphae at 3 μ.

The general development of *P. simplex* agrees in every way with the species already described, and the general structure of its mycelium very closely resembles some of these species, but it can easily be distinguished from them by its characteristic and definitely shaped sub-stomatal vesicle.

The spore on germination forms a delicate germ tube, the tip of which swells up to form the appressorium and then follows the sub-stomatal vesicle, which is at first a long sausage-shaped body, which always lays itself with its long axis parallel to the stomatal slit. At first (in shape) it is very much like *P. glumarum*, but it soon begins to differ from it in the following important points.

1. Infecting hyphae simultaneously spring from both ends of the vesicle.

These hyphae only measure from 3–3·5 μ across, and contain few nuclei, which are usually seen in pairs, whereas in *P. glumarum* the sub-stomatal vesicle only gives rise to a single infecting hypha from one end. From this hypha, which measures 10–12 μ across, the other hyphae arise, and these are always crammed with nuclei.

2. Soon after two or more pairs of infecting hyphae have *sprung* from the vesicle, it becomes divided transversely into two equal halves. Close to this partition wall, and on each side of it, a hypha then usually springs, and the vesicle may then become further sub-divided by transverse walls. (Figs. 35–37.)

Thus the sub-stomatal vesicle in *Uredo simplex* becomes multi-septate.

The Hyphae.

The hyphae closely resemble those of *P. Symphyti-Bromorum*, as described by Marshall Ward (33), both with regard to size, vacuoles, and septation.

As the hyphae begin to get older, they lose their protoplasmic contents, become very vacuolated, with a diminution in size of their contained nuclei, which are seen in the last stages of degeneration as small, round, dull, staining dots. Directly the Uredo pustule-formation begins, the mycelium that gave rise to it, including also the sub-stomatal vesicle, is seen to be quite empty and septate, except in the immediate neighbourhood of the pustule, where the hyphae are full of protoplasm and contain large and sharply staining nuclei.

In *P. simplex*, the long runner-like hyphae so characteristic of *P. Symphyti-Bromorum* and *P. glumarum* are not anything like so numerous or well developed, but single ones are found here and there running through the tissue of leaf as a simple thread for some distance

before they show any signs of branching. This is naturally what we would expect, for the pustules are always few, and scattered at great distances on the leaf.

Nuclei.

The nuclei closely resemble those of *P. Symphyti-Bromorum*. They are oval in shape, and nearly always seen in pairs, lying just one behind the other in the thin hyphae. They divide very rapidly by the direct method. A large nucleus becomes drawn out into a long oval-shaped body and is then gradually constricted at the middle, the two halves finally separate, regain their normal size and shape, and remain for some time in close proximity to one another.

Haustoria.

The fully-developed haustoria all appear to be of the shape shown in Fig. 44, but frequently this form is seen coiled on itself, as is shown by Marshall Ward (33) for *P. Symphyti-Bromorum*. Here, again, the hammer-headed form so common in *P. Symphyti-Bromorum* is very rarely seen.

Puccinia coronifera, Kleb.

This is the 'crowned rust' of the Oats; for some time it was included under the Crown Rust proper, *P. coronata*, Corda, but for reasons given below it must now be regarded as a distinct species.

Nielsen (28) first showed that the aecidiospores from *Rhamnus cathartica* when placed on *Lolium perenne* produced the uredospores, and eventually teleutospores, whereas aecidiospores from *Rhamnus Frangula* failed to infect *Lolium perenne*.

Plowright (30) found that the teleutospores from *Dactylis glomerata* and *Festuca silvatica* readily produced the aecidium on *R. Frangula*, and so he says that he thinks two species are confounded under the name *P. coronata*. He also adds the following interesting and important fact, which I also have noted during the past two years, viz. the fungus which occurs on *Lolium perenne* is accompanied by a profuse development of uredospores, but only in the autumn, from September to November, after which the teleutospores follow; whereas the fungus on *Dactylis* is an early summer species with a much less free development of uredospores. Klebahn (20) in 1892 showed that the teleutospores from *Lolium perenne* when sown on *Rhamnus cathartica* readily produced the spermogonia and aecidia, whereas similar sowings on *R. Frangula* failed.

On this account Klebahn (20) in 1892 insisted on the splitting up of the old *P. coronata*, Corda, into two species.

I. *P. coronata*, Corda. With its aecidium on *Rhamnus Frangula* (*Aecidium Frangulae*, Schum.), uredo- and teleutospores on *Dactylis glomerata*, *Festuca silvatica*, and other grasses.

II. *P. coronifera*, Kleb. With its aecidium on *Rhamnus cathartica* (*Aecidium Rhamni*, Gmel.). Uredo- and teleutospores on *Avena sativa*, *Lolium perenne*, and *Festuca elatior*.

There are six specialized forms of *P. coronifera*:—

1. *P. coronifera Avenae*, Erikss. Uredospores from Oats will infect only oats, not other grasses.

2. *P. coronifera Lolii*, Erikss. Uredospores from *Lolium perenne* will only infect *Lolium perenne*.

3. *P. coronifera Festucae*, Erikss. Uredospores from *Festuca elatior*, but not *Avena sativa*, *Alopecurus pratensis* (Erikss.). *Holcus mollis* and *H. lanatus* (Kleb.).

Further experiments are needed in the case of its transference to *Lolium perenne*, for Eriksson entirely failed to infect this latter, whereas Klebahn got a very fair percentage of infections on it.

4. *P. coronifera Holci*, Kleb. Uredospores from *Holcus mollis* and *H. lanatus* infect only these plants.

5. *P. coronifera Alopecuri*, Erikss. Uredospores from *Alopecurus pratensis* infect only *Alopecurus pratensis*.

6. *P. coronifera Glyceriae*, Erikss. Uredospores from *Glyceria aquatica* infect only *Glyceria aquatica*.

Uredo coronifera is easily distinguished in the field from *Uredo graminis* on Oats, by its light yellow-coloured pustules. These are of smaller size than those of *U. graminis* and are usually only found on the leaf, rarely on the stem.

The spores are round, yellow, and measure from 20–22 μ in diameter.

I have evidence to show that the Crowned Rust of Oats does considerable damage to the Oat crops in the Transvaal. A point of interest and one worthy of note is that this rust appears later in the season than *Uredo graminis*. As far as I am aware the aecidium has not been definitely shown in this country, but it should be mentioned that MacOwan in 1877 collected an aecidium on *Rhamnus prinoides* in Cape Colony.

According to Carleton this rust is widely distributed in the States, and is heteroecious on *Rhamnus lanceolata*.

The Histology of Uredo coronifera Avenae, Erikss.

The general development of the Fungus agrees in every way with the species already described, so that only the more important points will be mentioned here.

The appressorium (only the remains of which are seen in Figs. 38, 39) is very similar to that of *Uredo graminis*, but the attendant substomatal vesicle is of very different shape and gives rise at once to two infecting hyphae, one from each end of the vesicle. These in turn frequently branch dichotomously.

The haustoria, especially in the cells bordering on the vascular bundles, are of large size and cylindrical in shape. The pin-head type so common in the case of *Uredo graminis* is rarely met with.

Puccinia Sorghi, Schwein.

This rust is probably to be found wherever Maize is cultivated. In some parts of South Africa considerable damage has been wrought in the 'Mealie' crop through the agency of this parasite; but as a general rule little attention is paid to it.

Kellerman (19) has shown that the uredospores of *P. Sorghi* readily infect any and all of the 'agricultural species' of Maize, and that Teosinte (*Euchlaena luxurians*) was also a host for this species of rust, but that attempts to inoculate *Sorghum vulgare*, *Saccharum officinarum* and *Tripticum dactyloides* failed.

Recently Arthur (1) (2) has shown that this rust is heteroecious on *Oxalis cymosa*, successfully producing the aecidia on Oxalis from the teleutospores, and in turn infecting the Maize with the aecidiospores so obtained.

In this country MacOwan, as far back as 1879, found an Aecidium in Cape Colony on *Oxalis Bowei*, and quite recently I find that Burtt-Davy has collected an Aecidium in Swaziland on *Oxalis setosa*. Whether these two act as hosts for *P. Sorghi* remains to be seen.

The pustules of *Uredo Sorghi* are found on both sides of the leaf. When they are closely crowded, they frequently run together to form a large dark brown erumpent pustule.

The uredospores are globose, elliptical or ovate, slightly verruculose, and measure from 23–30 × 22–25 μ.

The sub-stomatal vesicle is somewhat triangular in shape when seen in longitudinal section (Figs. 40, 42), and gives rise to two or more infecting hyphae from the angles of the base.

In transverse section it is of the form shown in Fig. 43.

The hyphae are a little more robust than those of the other species described above, with the exception of *P. glumarum*, measuring as a rule from 4·5–5 μ in diameter.

The haustoria are frequently branched, as is shown in Fig. 41, and are constantly found in contact with, or carefully wrapping round, the nucleus of the host cells.

Summary of Results.

As the accompanying table will show, these different species of *Puccinia*, in the early stages of development of their Uredo mycelium exhibit morphological characters (seen especially in connexion with the formation of the sub-stomatal vesicle) which serve at once to distinguish them from one another.

The sub-stomatal vesicle is of a definite shape for each species. It may be non-septate, septate, or even multi-septate, and may give rise to one, two, or more infecting hyphae, according to the species. In some species a well-defined appressorium is present, in others it is not so apparent.

The vegetative hyphae of the different species closely resemble each other in size and general behaviour, except in the case of *P. glumarum*, where they are much thicker than any of the other forms, and contain a much greater number of nuclei.

The haustoria of some of the species are very distinctive, e. g. the hammer-headed form is one most commonly met with in *P. Symphyti-Bromorum*, whereas in *P. glumarum*, especially in the cells surrounding the vascular bundles, they are frequently very branched, while in the chlorophyll-containing cells the typical form of haustoria is that of a small club-shaped body.

Whether such slight morphological differences as are exhibited by the mycelia of *P. graminis* on Wheat and *P. Phlei-pratensis* are sufficient to warrant them specific rank, may at first sight seem doubtful. Consequently, I will leave the discussion of these points, until I treat of the so-called 'physiological species' which will, I think, have an important bearing on the matter.

Table showing Histology of Uredo Mycelia.

Species.	*Germ Tube.*	*Appressorium.*	*Substomatal Vesicle.*	*Hyphae.*	*Haustoria.*	*Nuclei.*
P. graminis on Wheat.	Two present as a rule, one generally becomes branched.	Well defined.	Cylindrical, non-septate, one end applies itself closely to the head of the guard cell, and gives off the infecting hypha. 27 μ in length. 9 μ in diameter.	Narrow, 3·5 μ in thickness.	Branched and unbranched.	Not numerous.
P. Phlei-pratensis on *Phleum pratense.*	Two present as a rule, one soon develops more quickly than the other.	Not so well defined as in *P. graminis.*	Cylindrical, non-septate. Gives rise to a single infecting hypha. 5–7 μ in diameter.	Very narrow and thread-like. Hardly 3 μ in thickness.	Mostly unbranched.	Not numerous.
P. Glumarum on Wheat.	Unbranched as a general rule.	No very definite structure, germ tube just becomes slightly swollen.	Somewhat oval in shape. Non-septate, only gives rise to single infecting hypha. 18–19 μ in diameter.	Broad and crammed with nuclei. From 10–19 μ in thickness.	Both branched and unbranched.	Very numerous.
P. dispersa on Rye.	Branched.	Well defined.	Cylindrical in shape, divided by transverse septum. Gives rise to two infecting hypha, one from each end of the cylinder. 11–14 μ in diameter.	From 3–5 μ in thickness, with nuclei arranged in single file.	Unbranched mostly, cylindrical in shape.	Not numerous.
P. triticina on Wheat.	Branched.	Well defined.	Spherical in shape, non-septate. Gives off single infecting hypha. 15 μ in diameter.	Narrow, 3·5 μ in thickness.	Branched and unbranched.	Not numerous.
P. Symphyti-Bromorum on *Bromus mollis.*	Generally branched.	Rather variable in shape.	Somewhat triangular in shape. Non-septate. Gives rise to one or two infecting hyphae.	Narrow and thread-like, about 3·5 μ in thickness.	Very variable in shape. Hammer-headed form very common.	Not numerous.
P. simplex on Barley.	Rarely branched.	Not well defined.	Cylindrical, multi-septate. May give rise to four or more infecting hyphae. 12–14 μ in diameter.	Narrow, 3·5 μ in thickness.	Unbranched, mostly of cylindrical shape.	Not numerous.
P. coronifera on Oat.	Branched or unbranched.	Not well defined.	Cylindrical, one septate, gives rise to two infecting hyphae, one from each end of the vesicle.	Narrow, 3·5 μ in thickness.	Unbranched, mostly of cylindrical shape.	Not numerous.
P. Sorghi on Maize.	Branched or unbranched.	Not well defined.	Triangular in shape, an oblique septum frequently present. Usually gives rise to two infecting hypha from the angles at the base of the triangle.	Narrow, from 4·5–5 μ in thickness.	Branched and unbranched.	Not numerous.

LITERATURE.

1. ARTHUR ('04): Bot. Gaz., xxxviii, 64, 1904.
2. ——— ('06): Jour. Mycol., xii, 1906.
3. BARCLAY: Journal of Bot., xxx, 45.
4. DE BARY ('63): Ann. des Sc. Nat., 4, xx.
5. ——— ('65): Monatsber. Akad. Berlin, p. 23.
6. BLACKMAN ('04): Ann. Bot., xviii, July.
7. BOLLEY ('06): Bull. 68 North Dakota, p. 627.
8. CARLETON ('99): Div. of Veg. Phys. and Path., Bull., xvi.
9. ERIKSSON ('94): Ber. Deutsch. Bot. Ges., xii, 310.
10. ——— ('96): Zeitsch. f. Pfl.-Krank., vi, 197.
11. ——— ('99): Ann. des Sc. Nat. 8, 9, 1899, p. 241.
12. ——— ('01): „ „ xiv, p. 101.
13. ——— ('02): „ „ Nos. 8 and 15.
14. ——— ('02): Ofv. Vet. Akad. Forh., p. 191.
15. ——— ('04): Kungl. Svenska. Vetenskaps. Akad. Handl., Bd. xxxviii, No. 3.
16. ERIKSSON and HENNING ('94): Zeitschr. f. Pfl.-Krank., iv, 141.
17. ——— ('96): Die Getreideroste.
18. ERIKSSON and TISCHLER ('04): Kungl. Svenska, Vetenskaps. Akad. Handl., Bd. xxxvii, No. 6.
19. KELLERMAN ('05): Jour. Mycol., January 11, 1905.
20. KLEBAHN ('92): Zeitsch. f. Pfl.-Krank., ii, 258.
21. ——— ('96): „ „ v, 332.
22. ——— ('97): „ „ vi, 36.
23. ——— ('98): „ „ viii, 338.
24. ——— ('00): „ „ x, 77.
25. ——— ('04): Ber. d. D. Bot. Ges., xxii, 255.
26. MÜLLER ('00): Vovl. Mitt. Bot. Centralbl., lxxxiii, 1900.
27. ——— ('01): Bot. Centr., x, 181.
28. NIELSEN ('77): Bot. Tidsskr., 3 R. 2, p. 26.
29. PLOWRIGHT ('82): Gard. Chron., p. 234.
30. ——— ('83): Grevillea, xi, 52.
31. SAPIN-TROUFFY ('96): Le Botaniste, p. 29.
32. MARSHALL WARD ('88): Ann. Bot., ii, p. 217.
33. ——— ('03): Phil. Trans., cxcvi, B.
34. ——— ('05): Ann. Bot., xix, Jan., p. 35.

EXPLANATION OF PLATES XL–XLIII.

Illustrating Mr. Pole Evans's paper on Cereal Rusts.

All the figures were drawn with the help of the camera lucida.

Figs. 1–6, 28–30, 38–43 were drawn with No. 2 eye-piece, and Zeiss Achromatic Apert. 1·30 Homog. Immers.

The remaining figures were drawn with Zeiss Apochromat. 2 mm. Apert. 1·40 Homog. Immers., using the No. 2 eye-piece for Figs. 13, 14, 16, 17, 19–24, the compensating ocular 8 for Figs. 7–11, 15, 18, 25–7, 31–7, 44, 45, and for Fig. 12 the compensating ocular 18.

PLATE XL.

Figs. 1–6. Uredo infection of *P. graminis* on Wheat.

Figs. 7–12. Uredo infection of *P. Phlei-pratensis* on *Phleum pratense*.

Fig. 1. Germination of uredospore of *Puccinia graminis*, with formation of appressorium over the stoma of wheat leaf. From a culture twenty-four hours old.

Fig. 2. Longitudinal section through stoma of wheat leaf, showing appressorium, substomatal vesicle, and infecting hypha of *P. graminis*. Four-day culture.

Fig. 3. Similar section of wheat leaf, showing substomatal vesicle and infecting hypha with haustoria. Four-day culture.

Fig. 4. Transverse section of wheat leaf, passing through a stoma, in which the appressorium and substomatal vesicle are seen, with the tip of an infecting hypha in the stomatal cavity. Four-day culture.

Fig. 5. Portion of hyphal filament with young haustorium.

Fig. 6. Typical haustoria found in the epidermal cells. Four-day culture.

P. Phlei-pratensis.

Fig. 7. Longitudinal section through stoma of leaf of *Phleum pratense*, showing stomatal vesicle and infecting hypha. From an eight-day culture.

Figs. 8, 9, and 10. Typical young haustoria of *P. Phlei-pratensis*.

Fig. 11. Older haustorium, its behaviour towards the nucleus of the chlorophyll-containing cell is very characteristic.

Fig. 12. Two vegetative nuclei in the process of direct division.

All the figures of uredo infection of *P. glumarum* on Wheat.

Fig. 13. Longitudinal section of leaf. Two spores are seen germinating at one stoma. From one the germ tube and appressorium are seen, with just a graze of the substomatal vesicle.

Fig. 14. The next section to the above showing the substomatal vesicle with its first infecting hypha, which has formed a haustorium in the first cell with which it has come in contact. From a five-day culture.

Fig. 15. Longitudinal section showing the oval substomatal vesicle just under a stoma, with the first infecting hypha, which has become septate at the tip previous to the formation of a haustorium. In connexion with some of the nuclei of the hypha, centrosomes are clearly seen. Third-day culture.

PLATE XLI.

Fig. 16. Transverse section across a stoma, showing the substomatal vesicle in section. Eight-day culture.

Fig. 17. Typical hyphae with their numerous nuclei. Eight-day culture.

Fig. 18. Large branched haustorium. The host cell nucleus, where the haustorium is almost in contact with it, is just beginning to show signs of a fragmentation. Eleven-day culture.

Fig. 19. Branched haustorium affecting host cell nucleus. The chlorophyll corpuscles are seen to have become rounded and swollen, in the act of breaking down. Eleven-day culture.

Figs. 20 and 21 show how narrow the hyphae may become in the intercellular spaces before spore formation. At the tip of the hypha in Fig. 21 a nucleus is just dividing. Both from a ten-day culture.

Fig. 22. Portion of a runner, the protoplasm has become much less dense and is very vacuolated. The nuclei are in a resting condition, and have distinct nuclear membranes.

Figs. 23 and 24. Common forms of haustoria.

Figs. 25–7, 33 and 34. Uredo infection of *P. dispersa* on Rye.

Figs. 28–30. Uredo infection of *P. triticina* on Wheat.

Figs. 31 and 32. Uredo infection of *P. Symphyti-Bromorum* on Bromus.

Fig. 25. Oblique longitudinal section of a stoma of a Rye leaf, showing a portion of a germ tube with its appressorium of *P. dispersa* just over the stomatal slit.

Fig. 26. Longitudinal section of a five-day culture of *P. dispersa* on Rye, showing the remains of the appressorium, the substomatal vesicle with its two infecting hyphae.

Fig. 27. Similar preparation to the above. In both cases the sub-stomatal vesicle has become divided by a transverse septum. Small centrosomes are seen in connexion with some of the nuclei.

Figs. 28 and 29. Longitudinal sections of Wheat leaf showing infection phenomena in *P. triticina*.

PLATE XLII.

Fig. 30. Transverse section across a stoma showing appressorium, substomatal vesicle, and infecting hypha with its haustorium of *P. triticina*.

Figs. 31 and 32. Longitudinal sections of stomata, showing infections with characteristically shaped sub-stomatal vesicles of *P. Symphyti-Bromorum*.

Fig. 33. Small portion of a typical hypha of *P. dispersa* with its nuclei. The tip of the hypha has just become transversely septate, preparatory to the formation of a haustorium. From a five-day culture.

Fig. 34. Typical haustorium of *P. dispersa*.

Figs. 35–7. Uredo infection of *P. simplex* on Barley.

Figs. 38 and 39. Uredo infection of *P. coronifera* on Oats.

Figs. 40–3. Uredo infection of *P. Sorghi* on Maize.

Fig. 35. Four-day culture of *P. simplex* on Barley, showing sub-stomatal vesicle with its infecting hyphae. The first transverse wall in the vesicle is just beginning to be formed.

Figs. 36 and 37. Both from the margins of Uredo pustules of *P. simplex* on Barley, taken from an outbreak in the field (and not artificially inoculated).

The sub-stomatal vesicles have become empty and divided by transverse septa. The hyphae are beginning to lose their protoplasm and become septate.

Figs. 38 and 39. Longitudinal sections of Oat leaf showing infection phenomena of *P. coronifera*. A single transverse septum is formed in each sub-stomatal vesicle, which gives rise to an infecting hypha from each end. Both five-day cultures.

Fig. 40. Infection of Maize leaf by *P. Sorghi*. The sub-stomatal vesicle is full of protoplasm, and not yet fully developed.

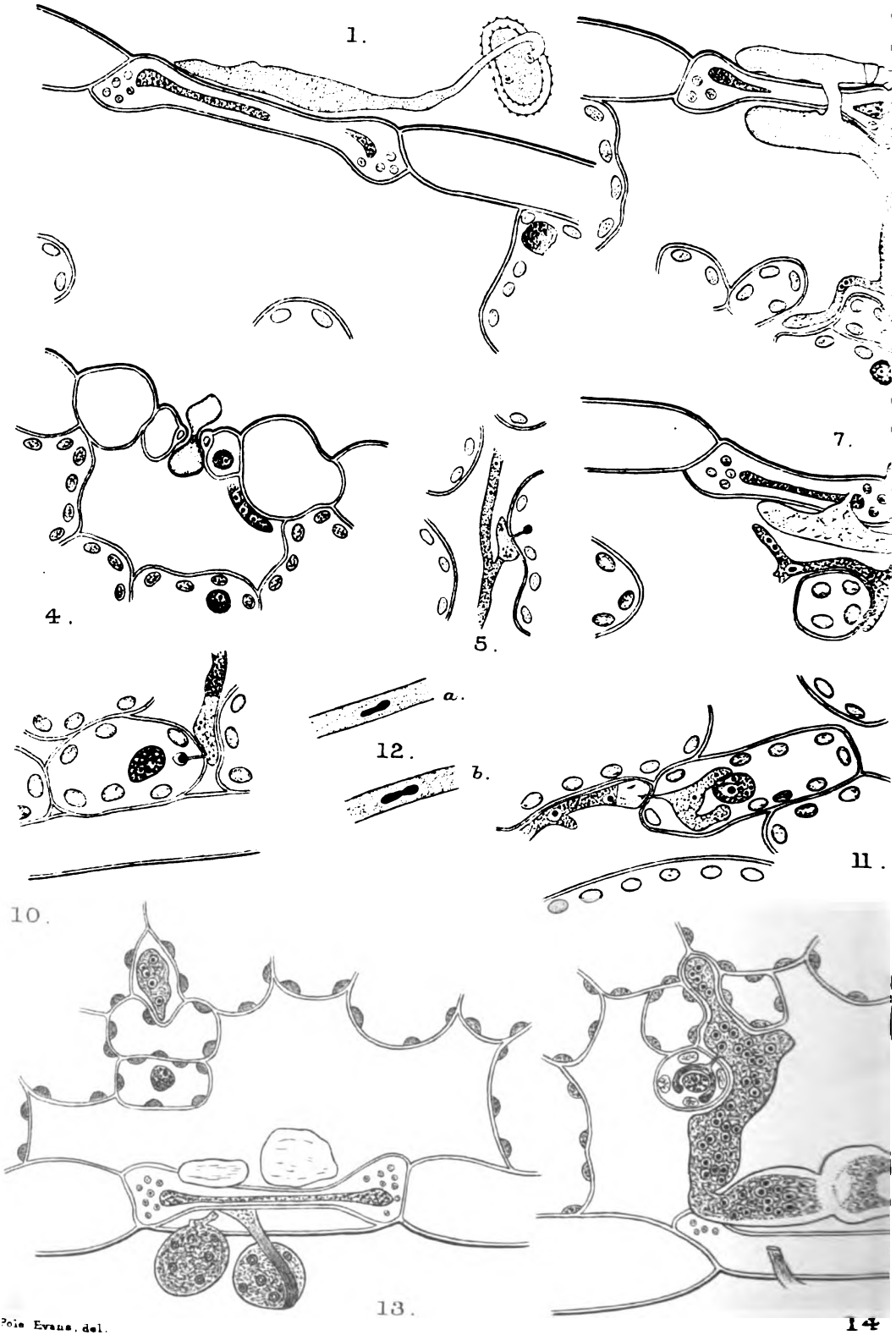
PLATE XLIII.

Figs. 41 and 42. Older infections of Maize leaf by *P. Sorghi*. Four-day cultures showing characteristically shaped vesicles with their infecting hyphae and haustoria.

Fig. 43. Infection of Maize leaf by *P. Sorghi*, showing shape of sub-stomatal vesicle in transverse section.

Fig. 44. Typical haustorium of *P. simplex* on Barley.

Fig. 45. Haustorium of *P. dispersa* on Rye.



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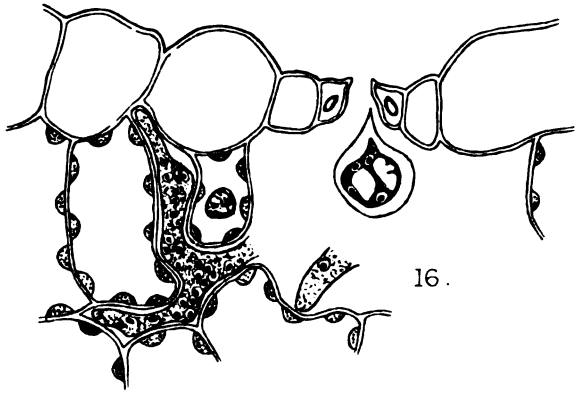

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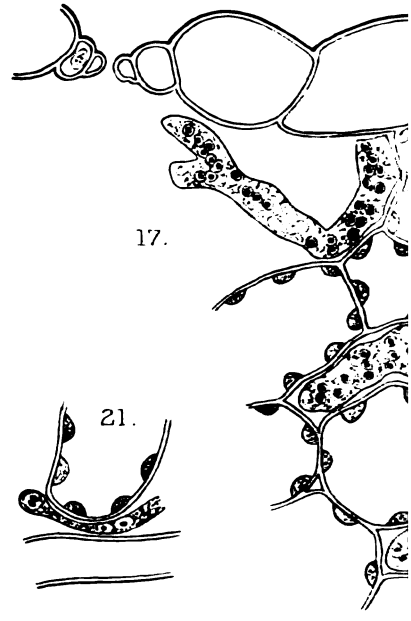

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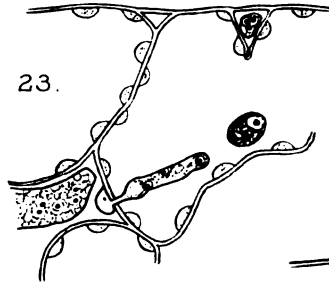

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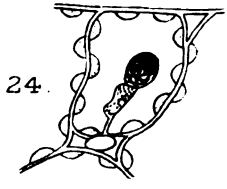

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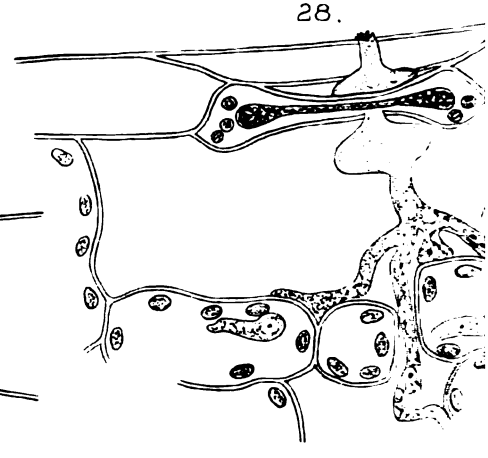

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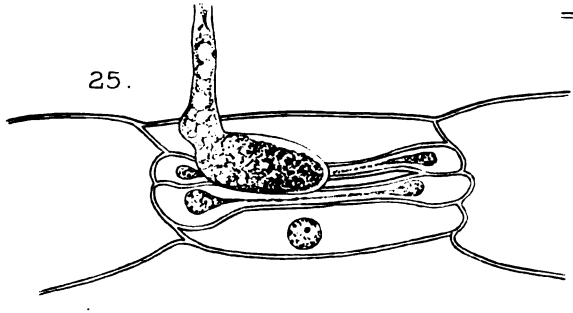

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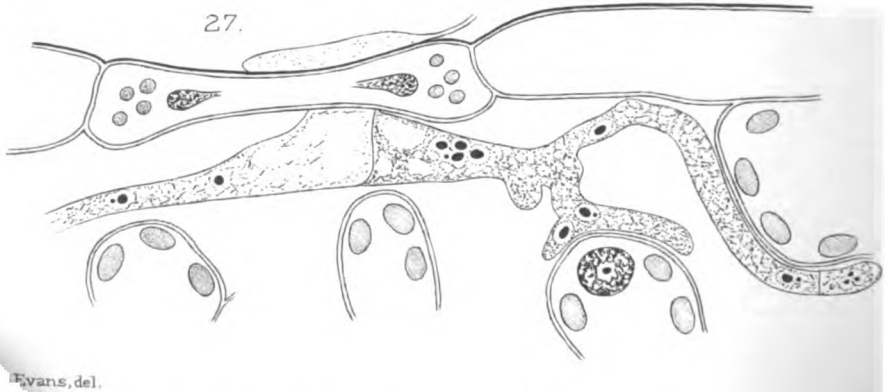

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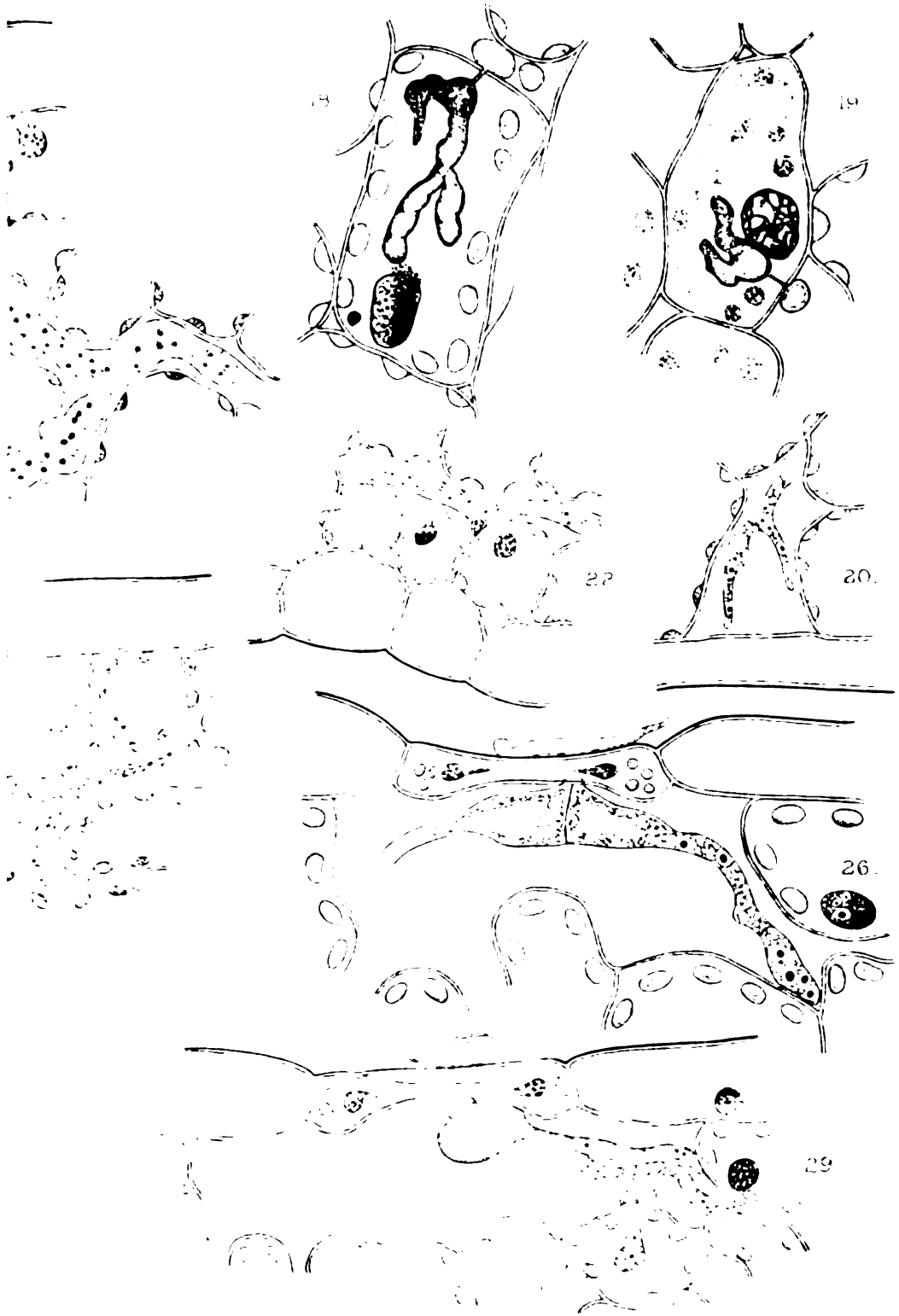
POLE EVANS — CEREAL RUSTS.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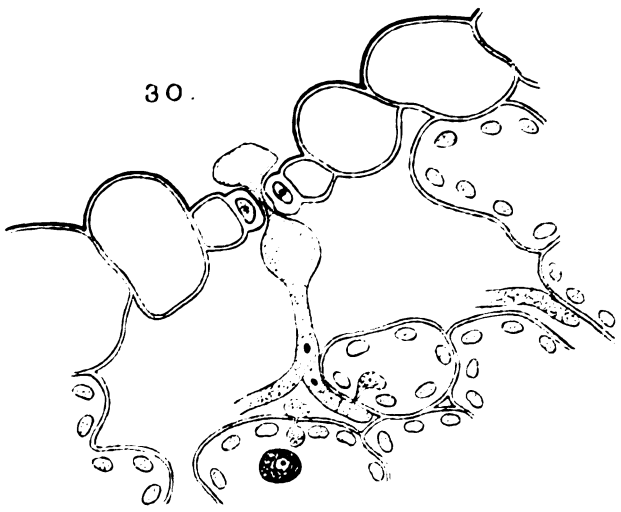
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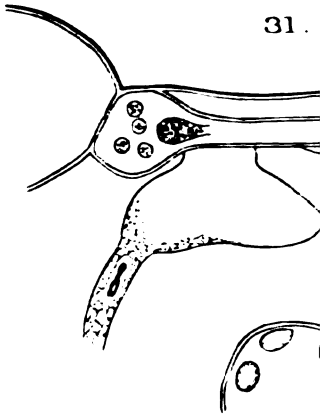
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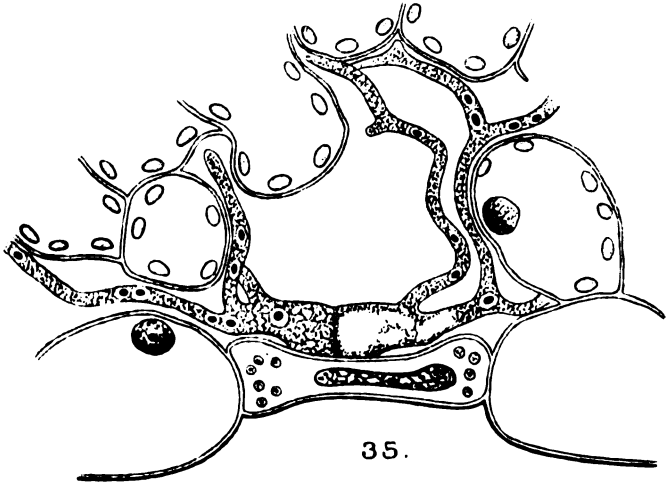
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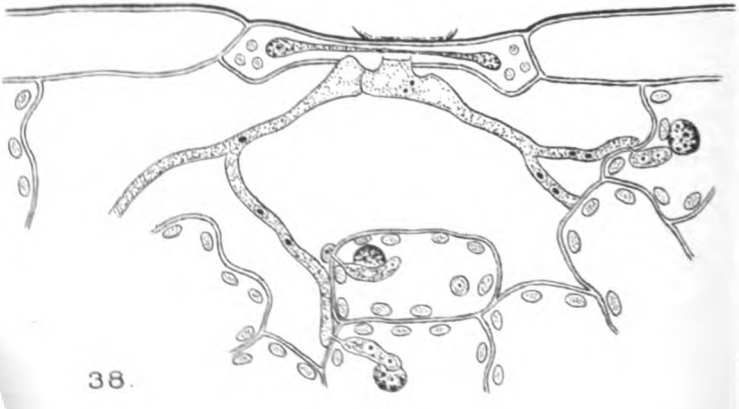
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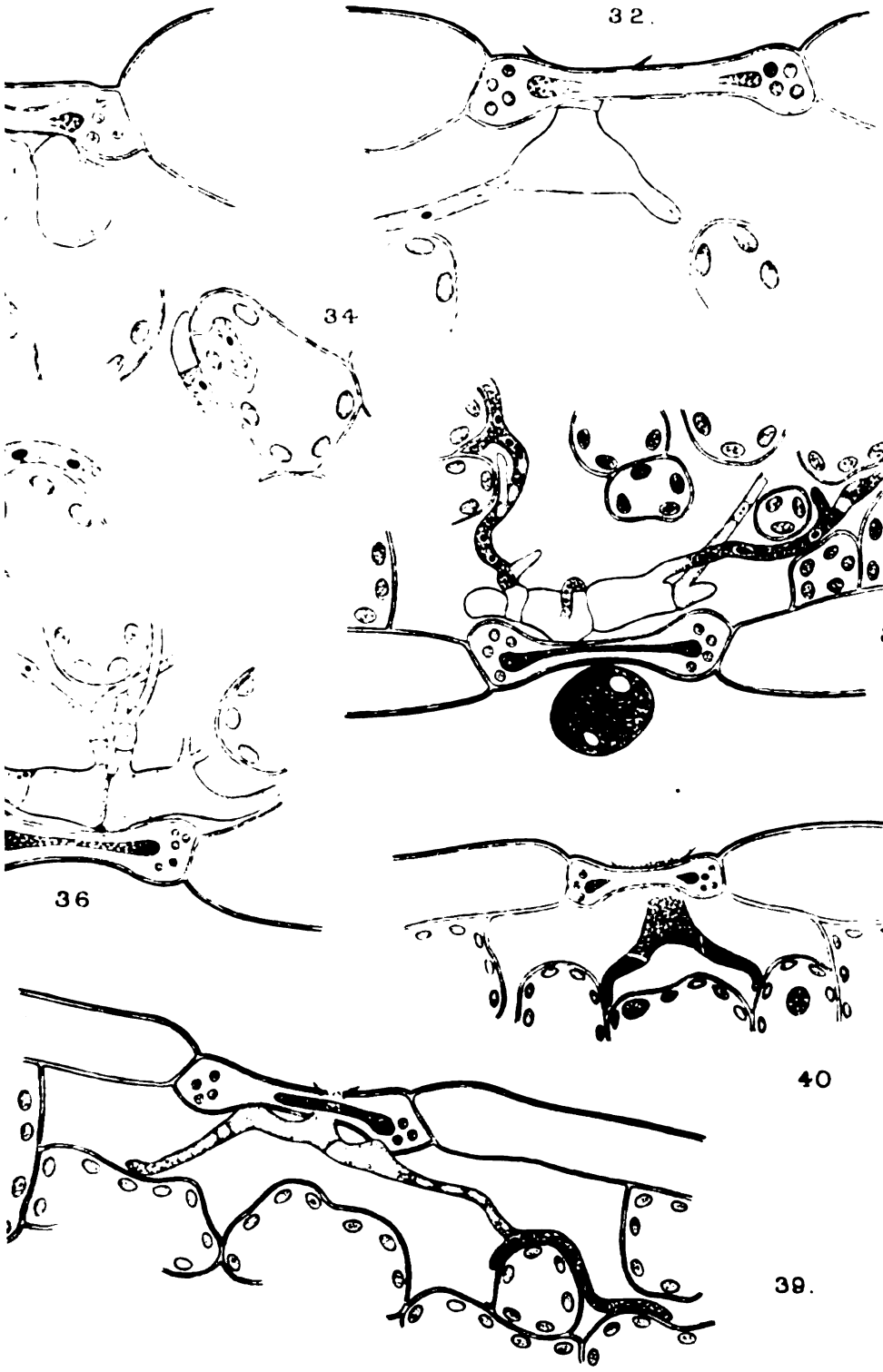

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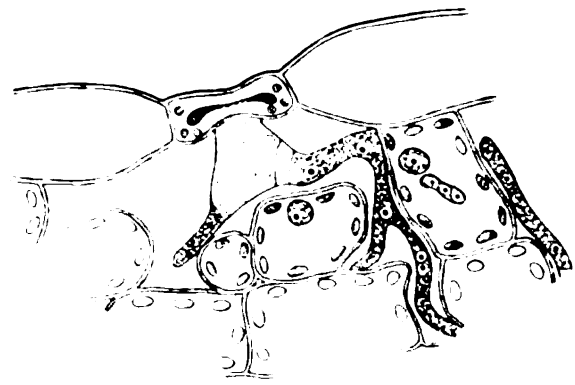

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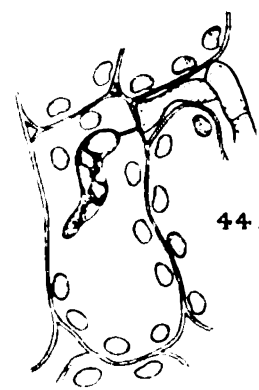

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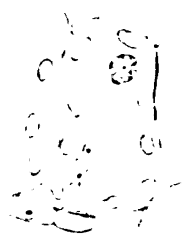

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POLE EVANS - CEREAL RUSTS.

Studies on some Javanese Anthocerotaceae. I.

BY

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With Plates XLIV-XLVI.

THE family Anthocerotaceae is a very isolated one, and its relation to the other Archegoniates is by no means clear. The single alga-like chromatophore, found in nearly all of them, the endogenously formed antheridia, and the characteristic sporophyte all distinguish them sharply from the Hepaticae with which they are usually associated. The proposal of Howe (the Hepaticae and Anthocerotes of California; Mem. Torrey, Bot. Club, VII, p. 9, 1899) to separate them from the Hepaticae, as a class Anthocerotes, has been accepted by the writer (Mosses and Ferns, 2nd Edition, 1905), and it is probable that this view will be maintained.

The researches of Hofmeister and the later works of Janczewski and others were mainly concerned with the common European species, *Anthoceros laevis*, but Leitgeb (Untersuchungen über die Lebermoose, Heft 5, 1879) also made a fairly complete study of several other species of *Anthoceros* as well as of species of two other genera, *Dendroceros* and *Notothylas*. Janczewski (Vergleichende Untersuchungen über die Entwickelungsgeschichte des Archegoniums, Bot. Zeit., 1872) showed that the early divisions of the archegonium were essentially the same as in the true Hepaticae, and Leitgeb confirmed his investigations. The latter writer thought that in *Notothylas* he had discovered examples where the sporogenous tissue arose from the endothecium, as in the true Liverworts, and he regarded *Notothylas* as intermediate between the Jungermanniales and the other Anthocerotaceae. This view, however, has not been confirmed by later researches (Mottier, Contributions to the Life History of *Notothylas*, Ann. of Bot., VIII, 1894; Campbell, Mosses and Ferns, 1st Edition, 1895).

Three genera are usually recognized—*Anthoceros*, *Dendroceros*, and *Notothylas*—which closely resemble each other in the characters of both gametophyte and sporophyte, and they are all obviously closely related. Of these three genera, *Anthoceros* is almost cosmopolitan. Schiffner (Hepaticae. Engler and Prantl, Nat. Pflanzenfam, 1 Th., Abt. 3, 1895)

[Annals of Botany, Vol. XXI. No. LXXXIV. October, 1907.]

gives seventy-nine species of *Anthoceros*, but a number of new species have since been described, and there are doubtless many more, as it is evident that the species have been quite superficially studied from many regions in the tropics where they abound. *Dendroceros* is credited with fifteen species, all of which are tropical, and *Notothylas* with nine, occurring both in tropical and temperate regions; but it is probable that the number of species in both of these genera is also greater than Schiffner indicates.

There are certain striking parallelisms in the character of the sexual organs and of the sporophyte between *Anthoceros* and the lower Pteridophytes, and also the Sphagnaceae, and the question arises whether these parallelisms indicate any true relationships. The writer has been inclined to believe that they do, although fully appreciating the difficulties in the way. The two greatest differences between the Anthocerotaceae and the lower ferns, like Ophioglossum, are the character of the antheridium and spermatozoids and the single chromatophore of the Anthocerotaceae. The former would allow a comparison with *Sphagnum* or with *Lycopodium*, but the single chromatophore of *Anthoceros* is very different from those of either of these forms. In all of the Anthocerotaceae hitherto described, a single large chromatophore is present in each cell, and this often contains a very distinct pyrenoid. The chromatophore thus resembles very much that of certain green Algae like *Ulva* or *Coleochaete*. It is not uncommon, however, to find in the larger interior cells of the thallus a division of the chromatophore, but hitherto no species was known in which the cells regularly contained more than one. In the summer of 1897 the writer collected in Jamaica an undetermined species of *Anthoceros* in which the superficial cells usually contained two chromatophores, but no further study was made of the plant at the time. This material was sterile, and could not be identified. No further discoveries were made until a recent visit to Java, where a similar form was discovered near Buitenzorg and later a second species (possibly more than one) was found at Tjibodas. About this time Goebel (Archegoniatenstudien, Flora 96, 1st Part; p. 195, 1906) noted the presence of multiple chromatophores in *A. giganteus* from New Zealand. As both of the Javanese forms, as well as *A. giganteus*, belonged to the section of the genus with spiral elaters and no stomata upon the sporogonium, it was thought that the Jamaica specimen might also belong to the same section.

Through the kindness of Dr. M. A. Howe, of the New York Botanical Garden, material of two American species of this section was secured—*A. Vincentianus*, Lehm. and Lindenb. from the West Indies, and *A. flavens*, Spruce, from South America—and in both of these the multiple chromatophores were found. This would seem to show that this character is probably common to all species of *Anthoceros* belonging to Gottsche's

third section of the genus, and, together with the other characters, would seem to constitute sufficient ground for separating these species as a distinct genus, as Leitgeb (loc. cit. p. 27) believed should be done. The discovery of the multiple chromatophores is important, as it breaks down one of the barriers separating the Anthocerotaceae from the other Archegoniates; but we still believe that the differences are sufficient to warrant the retention of the class Anthocerotes.

MEGACEROS, A NEW GENUS OF ANTHOCEROTACEAE.

Shortly after the writer's arrival in Java, in March, 1906, while on a botanizing excursion to the Tjiapus Gorge at the base of Mount Salak, near Buitenzorg, a number of species of *Anthoceros* were collected, and among them was one growing among mosses upon a fallen log. As this was a position very similar to that in which the West Indian specimens were collected, it was hoped that this might prove to be a similar form, and an examination after returning to Buitenzorg showed that such was the case. These specimens were fruiting abundantly, and it was at once evident that the plant belonged to the section with spiral elaters and no stomata, thus approaching *Dendroceros*. Later collections at Tjibodas showed that a similar species was not rare in the neighbourhood, and plants were repeatedly collected at various points from the immediate neighbourhood of the laboratory (altitude about 1,450 metres) to a short distance below Kandang Badak, which is about 2,500 metres high. A comparison of these specimens with those collected on the Salak showed that they were evidently specifically distinct, although much resembling the species from the lower level.

The Tjibodas material showed a good deal of variation as to habitat. Most commonly, as in the specimens first collected, the plants grew on rotten logs; but sometimes they were found upon the earth, and, in a few instances, upon boulders. The latter specimens had a thicker thallus than the others, but otherwise were not perceptibly different, and probably are not specifically distinct. A number of specimens with quite different chromatophores were found in the material when it was examined upon the writer's return to America, but these were sterile, and so could not be compared with the others. It is possible that they represent a third species, but at present this can only be conjectured.

The multiple chromatophores, together with the *Dendroceros*-like type of sporogonium, i. e. spiral elaters, green spores, and the absence of stomata, seems to warrant a separation of the group from the other species of *Anthoceros*, as Leitgeb suggested should be done. The name *Megaceros* is here proposed for all the species of this group. The name is selected on account of the very large size of the sporophyte in the commonest Javanese species. This in some instances reaches a length of 9 cm. and

possibly more, and *A. giganteus* is also characterized by its very long sporophyte. Stephani has described a species of *Anthoceros*, *A. Stahlii*, which evidently belongs to the same group as the species here described. The specimens were collected on Mount Gedeh, where the writer also collected much of his material. The original description was not accessible to the writer, but through the kindness of Dr. Howe a copy of the description was sent him.

The Tjibodas form, or at least the commonest one, agrees in many respects with Stephani's description of *A. Stahlii*, and may possibly be the same, but the thallus in the writer's specimens is very much thicker, and the spores and especially the elaters much larger than Stephani describes for *A. Stahlii*. For this species, the name *Megaceros Tjibodensis* is proposed. The species from Mount Salak we shall call *M. Salakensis*. The important points of difference between *Megaceros* and *Anthoceros* are the multiple chromatophores of the former, the absence of stomata from the sporogonium, the solitary antheridium, spiral elaters, and green spores. In all of these respects, except the character of the chromatophores, *Megaceros* resembles *Dendroceros* more than it does *Anthoceros*; but the form of the thallus and the apical growth, at least in all of the forms examined by the writer, are like those of a typical *Anthoceros*. It may be noted here that *Anthoceros Pearsoni*, Howe, a common Californian species, has solitary antheridia, and it is interesting to note that the inner thallus cells very commonly show a double chromatophore. It is, therefore, to a certain extent intermediate between the typical species of *Anthoceros* and *Megaceros*.

Both *M. Tjibodensis* (Pl. XLIV, Fig. 1) and *M. Salakensis* (Fig. 4) have a relatively large thallus, which in the former may reach a length of 5 cm. or more, with a breadth of about 1 cm. Of the different forms which seem to belong to *M. Tjibodensis*, the largest were growing upon boulders and upon the earth. These have a thicker thallus than the typical form shown in Fig. 1, and may possibly be specifically distinct. In the type the thallus is rather thin, with a conspicuously lobed and strongly laciniate margin (Fig. 6). The laciniate character is somewhat less marked in the stouter rock form (Fig. 7).

M. Salakensis is somewhat smaller in all its parts, and the margin of the thallus is lobed, but does not show the laciniate margin so marked in *M. Tjibodensis* (Fig. 8). The sporogonium in *M. Salakensis* is also very much shorter than in *M. Tjibodensis*. In the latter it may reach a length of 9 cm. (see Fig. 3), while in *M. Salakensis* the largest specimens found were less than half as long (Fig. 5). In the latter species also, the ripe sporogonium opens along one suture instead of dividing into two valves, which is the rule in *M. Tjibodensis*. In this respect, as well as in some others, *M. Salakensis* resembles *Dendroceros* more nearly than does

M. Tjibodensis. In both species the usual Nostoc colonies occur, but these are not especially noticeable.

The antheridia are produced singly in the usual chamber, and are large enough to be seen with the naked eye, in some cases having a diameter of about 170 μ. Stephani states that in *M. Stahlii* they do not occur upon the same branches of the thallus as the archegonia, but in all forms studied by the writer they occurred upon the same shoot. The plants are markedly proterogynous, however, unlike most species of *Anthoceros*, where the antheridia are formed first. It may have been the overlooking of this fact that led Stephani to suppose that they were borne upon special branches.

The Chromatophores.

A careful study of the chromatophores of both of the Javanese species was made. These are most different from the ordinary *Anthoceros* type in *M. Tjibodensis.* In most species of *Anthoceros*, as is well known, each cell contains a single large flat chromatophore, in the centre of which is a very distinct pyrenoid, the whole closely resembling the chromatophores of many green Algae, especially many Confervoideae, like *Stigeoclonium* or *Coleochaete.* A careful study of *M. Tjibodensis* shows that not only is the single chromatophore replaced by several, sometimes as many as twelve, but all trace of a pyrenoid has disappeared, so that the chromatophore is in all respects like that found in the other Archegoniates. The pyrenoid also seemed to be absent from the chromatophore of *M. Vincentianus* and *M. flavens*, of which dried specimens were examined; but in *M. Salakensis* (Figs. 13 to 17) there was an aggregation of small starch granules about a central area in the larger chromatophores, which had the appearance of a pyrenoid, although it was much less definite than is usually the case in most species of *Anthoceros.*

M. Tjibodensis, although in a few of the superficial cells a single chromatophore may occur, in most cases has from two to four, while the number is much greater in the inner cells of the thallus. In the superficial cells of the upper side (Fig. 9) there are most commonly two chromatophores. Sometimes there is a single one and sometimes three. In the superficial cells of the ventral side the number ranges from two to four (Fig. 10), and they are smaller, but otherwise resemble those of the dorsal surface. They are in both cases thin flat plates, showing a uniform finely granular appearance, but no trace of anything like a pyrenoid, nor were large starch granules observed. In the cells immediately below the surface there are usually four smaller chromatophores, while in the large inner cells (Fig. 11) they are much more numerous, but of smaller size, and often quite round, closely resembling the typical chlorophyll bodies of higher plants. The nucleus (*n*) in all the cells is very small,

and not at all conspicuous, but may easily be demonstrated. Fig. 12 shows a cell from the interior of the thallus of a form which was growing with the typical *M. Tjibodensis*. The specimens were not fertile, and no comparison could be made with the sporogonium of *M. Tjibodensis*, so that it was not possible to determine certainly whether it was specifically distinct, but the very different form of the chromatophores would indicate that it is a different species. There were seldom more than four chromatophores present even in the largest cells, and these were flattened and irregular in outline, and were more or less completely joined by fine protoplasmic filament, presenting a very different appearance from the small rounded and isolated chromatophores of the typical specimens.

M. Salakensis differs a good deal from *M. Tjibodensis* in the character of the chromatophores. In the cells of the upper surface there is usually but a single chromatophore (Fig. 13), but this is often constricted, and may be completely divided into two. In the cells of the ventral surface two distinct chromatophores are generally present. These show a central area which is surrounded by numerous starch granules, and there are indications of a pyrenoid, but as we have seen, it is much less evident than in the typical Anthocerotaceae, where it is often very conspicuous. The cells below the epidermal layer contain usually two smaller chromatophores (Fig. 15), which also show the pyrenoid-like centre body. The inner cells of the thallus (Figs. 16 and 17) contain from four to six small rounded chromatophores without any central body, which are connected by delicate protoplasmic threads into a sort of chain.

Through the kindness of Dr. M. A. Howe, dried material of two species from tropical America was secured. An examination of these showed that they also possess multiple chromatophores. These were *A. Vincentianus*, Lehm. and Lindenb., from Martinique, and *A. flavens*, Spruce, from the Peruvian Andes. In both of these species (Figs. 18 and 19) the inner cells contained two to four chromatophores, while the superficial cells had usually but a single one, although it was often deeply constricted, and occasionally divided completely in two. From this dried material it was not possible to make a study of the structure of the chromatophore beyond noting that apparently no pyrenoid was present, in which respect they seem to agree with *M. Tjibodensis*.

While in most other Archegoniates the chromatophores are small and numerous, there are a number of exceptions which approach the condition found in *Megaceros*. The writer has found, as an exceptional occurrence, large flat chromatophores in the prothallial cells of *Osmunda cinnamomea* (Campbell, On the Prothallium and Embryo of Osmunda. Ann. of Bot., VI, 1892), and in a species of Cyathodium collected in Java, apparently somewhat different from the common *C. foetidissimum*, the chromatophores were relatively very large, and in some cases there were only four in a cell.

The chromatophores in the assimilative cells of *Targionia hypophylla* are also few in number, and relatively large. The single chromatophore found in some of the species of *Selaginella* may be also mentioned in this connexion. These instances will serve to show that the chromatophores of the Anthocerotes are not so radically different from those of the other Archegoniates as has been generally assumed.

Structure of the Thallus.

The general form of the thallus in both *M. Tjibodensis* and *M. Salakensis* resembles that of a typical *Anthoceros*, and in neither of these species could any trace of a mid-rib such as occurs in *Dendroceros* be seen. In *M. giganteus*, however, Goebel (loc. cit., p. 195) states that the thallus is costate, although not so distinctly so as in *Dendroceros*, which it evidently resembles in habit. This species showed two to four chromatophores, which, like those of *M. Salakensis*, were often joined together. No statement is made by Goebel as to the character of the apical cell, which in *Dendroceros* is quite different from that of *Anthoceros*.

The thallus in both of the species under discussion is decidedly thicker than that of *A. Stahlii*, to judge from Stephani's description. He states that in the latter the thallus is six cells thick in the middle and only three cells thick in the wings. The specimens of the typical form of *M. Tjibodensis* are from eight to ten cells thick in the middle, diminishing very gradually toward the margin to four or five. In no case was any part of the thallus seen with less than four cells. The larger form, growing on boulders, in some cases showed twelve cells in the central region. *M. Salakensis* is slightly thicker than the typical *M. Tjibodensis*—usually nine to ten cells in thickness. In both species, as in *Anthoceros*, the superficial cells are flattened, and the chromatophores larger than in the inner ones. The sub-epidermal layer is often quite well defined, the cells being intermediate in size between the shallow superficial ones and the four or five layers of large central ones.

The apical growth is entirely like that of *Anthoceros* and the large initial cells show a regular succession of dorsally and ventrally arranged segments (Fig. 20). Each segment divides into outer and inner cells, and from the former in the fertile branches the sexual organs arise. In a few exceptional cases an approach to the condition found in *Dendroceros* was noted. In the latter genus the initial cells in vertical section appear semicircular in outline, and segments are cut off from the inner face which extend the whole depth of the thallus, and are subsequently divided by the median wall into a dorsal and a ventral portion. Fig. 21 shows a section of the apex of the thallus of *M. Tjibodensis*, in which there is an approach to the condition found in *Dendroceros*. No intercellular spaces are present

in the thallus of either species, but there are numerous large cells filled apparently with a mucilaginous substance which stains very strongly.

The Sexual Organs.

Both of the species of Megaceros under consideration are monoecious. Stephani (loc. cit.) states that in no cases did he find in *M. Stahlii* antheridia and archegonia upon the same branch, and thinks it may be dioecious. In both *M. Tjibodensis* and *M. Salakensis* the two sorts of organs are found upon the same branch, but, unlike most species of *Anthoceros*, the archegonia are developed first, and one may find fresh antheridia upon the younger parts of branches which bear advanced sporogonia. It may be that owing to this marked proterogeny Stephani failed to find antheridia and archegonia together.

The Antheridium.

The antheridia, as has already been noted in *M. Vincentianus* (?) (Leitgeb, loc. cit., p. 17) and *M. Stahlii* (Stephani, loc. cit.), occur singly, and in this respect resemble those of *Dendroceros*. As we have already stated, however, there is one species of typical *Anthoceros*, *A. Pearsoni*, in which solitary antheridia also occur, and this fact, together with the frequent doubling of the chromatophores in the inner cells, suggests an approach to *Megaceros*.

The study of the development of the antheridium is attended with some difficulty owing to the mucilage developed about it in the chamber where it is formed. This mucilage evidently interferes with the penetration of fixing agents, and much of the material that was examined was found to have the younger antheridia so shrunken as to be quite useless for study. This was specially the case with specimens treated with 1 per cent. chromic acid, which otherwise proved the best fixing agent. Specimens fixed with acetic alcohol (alcohol, 90 per cent.; acetic acid, 10 per cent.) gave much better results, although for the study of the archegonia and embryo the chromic acid yielded much better preparations.

The early stages of the antheridium closely resemble those of *Anthoceros Pearsoni* (see Campbell, Mosses and Ferns, 2nd Edition, Fig. 57). The superficial cell from which the antheridium arises, as in all other Anthocerotaceae that have been studied, divides by a periclinal wall into an outer and inner cell, the latter in *Megaceros*, as in *Dendroceros* and *Anthoceros Pearsoni*, becoming at once the single antheridium. In the other species of *Anthoceros* and in *Notothylas* the inner cell divides by longitudinal walls into (usually) four cells, each of which develops into an antheridium. Before any divisions take place in the young antheridium, there begins to develop the cavity or chamber in which the antheridium

lies. The cells adjoining the mother-cell of the antheridium withdraw slightly from it, apparently owing to a mucilagenous degeneration of the inner layers of the cell walls. This mucilage forms a strongly staining substance outlining the young antheridia in stained sections, and with the growth of the tissue near it the chamber rapidly enlarges and becomes very conspicuous (Figs. 24, 25).

In the youngest stage that could be recognized (Fig. 23) the mother-cell had divided into a small basal cell and a large terminal one. Whether this division always occurs is doubtful, but usually traces of the basal cell could be made out in the younger antheridia (Fig. 24 b). The first wall in the antheridium itself is longitudinal (Fig. 24), and this is probably in most cases at least followed by a second longitudinal wall intersecting the first. Following this, transverse walls arise that separate the pedicel from the upper portion. There next arise periclinal walls in each of the upper segments, by which the inner cells that develop into the sperm cells are separated from a single layer of parietal cells. Each of the latter finally develops a conspicuous chromatophore, as in other cases. The further divisions in the central cells follow with a good deal of regularity, and the limits of the early cell divisions are evident, even in the nearly ripe antheridium where the mass of almost cubical spermatocytes is divided into blocks corresponding to the early divisions of the central cell, a phenomenon which is of common occurrence in many other Bryophytes. The small size of the spermatozoids makes them unfavourable subjects for study of spermatogenesis, and no attempt was made to follow out the details. In the adult antheridia the slender curved body of the spermatozoid can be readily seen, but the blepharoplast, which is presumably present, could not be seen. Free spermatozoids were found to resemble those of ordinary Bryophytes, having the usual two cilia, but beyond demonstrating this point no further study was made of them.

The two species studied differed a good deal in the form of the antheridia. In *M. Tjibodensis* (Fig. 31) the mature antheridium is nearly globular, and has a short stalk inserted near the centre of the floor of the antheridial chamber. In *M. Salakensis* the insertion of the pedicel is toward the front of the antheridial chamber, not infrequently actually on the anterior wall of the cavity, and the antheridium lies almost horizontal, as it does in *Dendroceros* (Fig. 29). The pedicel is much longer than in *M. Tjibodensis*, and strongly bent, in this respect also resembling *Dendroceros*, although it never reaches the extreme length found in the antheridium of the latter. The pedicel in both species examined has ordinarily four rows of cells, but it is possible that sometimes, as is usually the case in *Dendroceros*, there may be only two rows. Rarely two antheridia occur in a single chamber.

Leitgeb (loc. cit., p. 19) has called attention to the occasional occur-

rence of superficial antheridia, but he recognizes that these are not normal cases. Two instances of a condition approaching this were seen in the course of these investigations. One of them is shown in Fig. 27, where the cavity containing the antheridium was open on one side so as to expose the top of the young antheridium. Apparently in this instance the primary cover cell of the antheridial cavity had been very early pulled away from the adjacent surface cells and the cavity thus became open, and no doubt, by the further lateral growth of the superficial cells, the antheridium would soon have stood in a shallow depression such as Leitgeb describes in the specimens seen by him. More recently Lampa (Untersuchungen an einigen Lebermoosen. Sitzungsber. der Kais. Akad. der Wiss., Wien, cxi, pp. 477, 489, 1902) has described exogenously-formed antheridia in *Anthoceros*; but Howe has criticized her work, and thinks that the structures described as antheridia were tubers, as the figures do not agree with the structure of normal antheridia (Howe, Torreya, iv, p. 175, 1904).

From a study of the development of the antheridium it is quite impossible to say whether or not the endogenous origin is primary or secondary, nor do the exceptional cases where the origin is superficial throw much light on the question. Such a condition as that shown in Fig. 27 is not unlike what is found in *Sphaerocarpus* or *Riccia*, where the antheridium is surrounded by an involucre; but whether this is any indication of a possible relationship between these forms and the Anthocerotaceae is another question. There can be no doubt that in all the Anthocerotaceae the antheridia are normally of endogenous origin.

The Archegonium.

The researches of Janczewski (loc. cit.) and those of Leitgeb, show that although the form of the archegonium is apparently quite different from that of the true Hepaticae, nevertheless in its essential structure it agrees closely with the other Bryophytes. All of the genera are much alike in the development of the archegonium, and *Megaceros* shows no marked differences when compared with the others. The youngest stages are not easily recognizable, as the archegonium does not project at all above the level of the thallus at first, and the mother-cell is not usually markedly different in appearance from the neighbouring cells (Fig. 20 ♀). Transverse sections of the young archegonium (Fig. 38) show that the axial row of cells is cut out by three intersecting walls as in the typical bryophytic archegonium, and longitudinal sections of the younger stages (Figs. 33–6) present an appearance not very different from similar sections of archegonia of true Hepaticae, except that in the latter the whole archegonium is free, while in the Anthocerotaceae only the upper surface is exposed. Nevertheless the limits of the neck cells are pretty well

defined, and in cross section (Fig. 39 a) the archegonia usually show the same number, six, that is characteristic of the lower Hepaticae.

The first division of the axial cell is transverse (Fig. 33) and usually there are no longitudinal walls formed; but in some cases the outer cell divides by a longitudinal wall (Fig. 34) before any further transverse divisions appear. In such cases the neck canal-cells are consequently much narrower than usual, and the central cell relatively broader. In most cases the breadth of all the cells of the axial row is approximately equal. The next transverse division is in the outer cell, and divides it into the cap-cell, and the primary neck canal-cell. The former divides by a vertical wall into two nearly equal cells, which may remain undivided, or one or both of them may divide again, so that the final number of cap-cells is two to four. The canal-cell divides by a series of transverse walls into four or sometimes five cells. In no case was a larger number found. Janczewski states that as many as twelve neck canal-cells may be formed in *Anthoceros laevis*, but the writer has never found more than six in any species of *Anthoceros*, *Notothylas*, or *Dendroceros* that he has studied. As in other cases that have been observed, the cover cells are thrown off when the archegonium opens, and the upper neck cells which project considerably above the thallus diverge more or less. As in the other Anthocerotaceae the central canal-cell is nearly or quite equal in size to its sister cell, the egg (Figs. 37, 40). No marked difference was observed between the archegonia of the two species studied. Of the three genera that have been examined hitherto, *Notothylas* has the axial cells relatively broadest, approaching in this respect the lower Pteridophytes. *Dendroceros* is intermediate between *Notothylas* and *Anthoceros* in the form of the archegonia, and *Megaceros* resembles it closely in this respect.

The Embryo.

The very small size of the spermatozoids makes the Anthocerotaceae unsuited to a study of the details of fertilization, and no attempt was made to investigate these in *Megaceros*. The egg-cell at the time of fertilization does not fill the central cavity, and its nucleus is small. After fertilization it grows until it fills the venter before the first division occurs.

In the early divisions of the embryo *Megaceros* more nearly resembles *Dendroceros* than it does *Anthoceros*. As in both of these forms the first wall is longitudinal and the second transverse; but whereas in *Anthoceros* the transverse walls are below the middle of the young embryo, this being very marked in *A. fusiformis* (Mosses and Ferns, 2nd Edition, Fig. 69), in *Dendroceros* they are nearly median, and the young embryo is divided into nearly equal quadrants. The same is true in *Megaceros*, although the two lower quadrants are slightly smaller (Figs. 41, 48). Of the two species,

M. Tjibodensis and *M. Salakensis*, the former (Fig. 41) has a broader embryo, and the lower tier of cells very early begins to send out the root-like outgrowths which become so prominent a feature of the foot in the older embryo. This early development of these root-like processes was noted by Leitgeb in *M. Vincentianus*. The next divisions are vertical ones, and these octant walls are followed by a second series of transverse walls, as in *Anthoceros* and *Dendroceros*. Both of the lower tiers form the foot, as in *Anthoceros*, but the differentiation into amphithecium and endothecium extends almost to the base of the foot. In the lower tier of cells the divisions are less regular and this seems to be especially the case in *M. Salakensis* (see Fig. 50 b), where the quadrant divisions may be only very imperfectly indicated.

The formation of the columella or endothecium and the outer tissue or amphithecium follows much as in the other forms that have been investigated, but this can be clearly traced to the lowest tier of cells in *Megaceros*, while in *Anthoceros* it is not usually evident below the base of the uppermost tier which marks the boundary of the foot. The cutting off of the endothecium may in some cases apparently be brought about by periclinal walls directly, but to judge from a study of series of transverse sections (Fig. 51) these anticlinals may sometimes at least be formed before the development of the periclinal walls which finally delimit the endothecium. This is very like what is sometimes found in some mosses, e.g. Funaria, where there is not absolute uniformity in the succession of walls in the young segments of the embryo.

While the very young embryo of *M. Tjibodensis* is broader than the corresponding stage of *M. Salakensis*, this is not true in the latter stages, where in the latter species the embryo is noticeably broader at the base than in *M. Tjibodensis*. This can be seen by comparing Figs. 43, 52 with Figs. 49, 55. In the first species (Fig. 52) the young sporogonium is slightly constricted, while in *M. Salakensis* the base is broader than the region above it.

In the differentiation of the columella and in the origin of the sporogenous tissue, the two species agree closely and resemble *Dendroceros* more than they do *Anthoceros*. Comparisons of longitudinal and transverse sections show very clearly the origin and extent of these tissues. Before the cutting off of the sporogenous layer (Figs. 49, 51) the endothecium (columella) forms a cylindrical mass of tissue occupying the centre of the embryo and surrounded by a single layer of amphithecial cells. It can be traced to the base of the second tier of cells, thus including the upper part of the foot. The latter is clearly limited by the second transverse walls in the young embryo, and there is very little displacement of these walls, so that there is a sharp line between the foot and the base of the capsule. In *Anthoceros* (Campbell, Mosses and Ferns, 2nd Edition, Fig. 70) the

sporogenous tissue does not extend to the base of the capsule, but there is a clearly defined basal zone where it is absent. In *Megaceros*, on the contrary, the sporogenous layer can be traced to the line dividing the foot from the capsule. In this respect *Megaceros* exactly resembles *Dendroceros* (Campbell, loc. cit., Fig. 79). As in all the other Anthocerotaceae the sporogenous layer arises from a series of periclinal walls in the amphithecium, and there is thus formed a single layer of cells investing the columella (Figs. 43–5). These do not form simultaneously (see Fig. 43), but very soon the sporogenous layer is complete and is then seen to extend to the base of the capsule.

The columella of the young embryo seen in cross section forms a nearly regular square of four cells. Subsequently, these are further divided (Fig. 45 b), and in the older sporophyte there may be sixteen cells arranged in regular quadrant fashion, or not infrequently (Fig. 58) there may be further divisions in some of the cells. This also recalls the larger species of *Dendroceros*. In longitudinal sections there usually appear four rows of cells in the columella except toward the apex, where the columella becomes narrow, this being especially marked in *M. Tjibodensis*, where it tapers to a point (Fig. 52).

While in the early development of the embryo and in the details of the adult sporogonium *Megaceros* most nearly resembles *Dendroceros*, the sporogenous tissue is much more extensive and may best be compared with that of *Notothylas*. In *Dendroceros* and in some species of *Anthoceros* the sporogenous layer remains simple throughout nearly its whole extent. In the larger species of *Anthoceros*, e. g. *A. fusiformis*, it becomes double, while in *Notothylas* it is three to four cells thick, occupying relatively a very much larger part of the sporophyte. In the extent of the sporogenous tissue *Megaceros* (Figs. 54, 57) is to some extent intermediate between *Anthoceros* and *Notothylas*. When the divisions are complete it is three to four cells in thickness, but the cells are smaller than in *Notothylas*, and hence the mass of sporogenous tissue is relatively less; but it is very decidedly greater than in any species of *Anthoceros* that has yet been studied.

While in *Anthoceros* the portion of the sporogenous layer above the apex of the columella has the same thickness as that at the sides, there is in *Megaceros* the development of a considerable amount of sporogenous tissue in the amphithecial cells lying above the layer in contact with the columella (Figs. 53, 56). This is especially conspicuous in *M. Tjibodensis*, where it is quite as marked as it is in *Notothylas*. A trace of this increase of the sporogenous tissue at the apex of the sporogonium is also seen in *Dendroceros*, but it is very much less marked. The development of sterile tissue in the apical region of the sporogonium of *Megaceros* is also greater than in the other genera.

The sporogonium may become very long, this being especially so in *M. Tjibodensis*, where it may attain a length of 9 cm. or possibly more. In *M. Salakensis* it is much shorter, but in both it is stout and the amount of sterile tissue large. To correspond with this development of the assimilative tissue the foot becomes very large, and at an early period develops extensively branched rhizoid-like outgrowths (Figs. 42, 47), which penetrate between the adjacent cells of the thallus. Sections through the older sporogonium show the different stages of development of the sporogenous tissue, as there is of course the same basal zone of meristem by which the growth of the sporogonium is brought about. Transverse sections, where the sporogenous layer is first clearly differentiated (Fig. 58), show the square columella, its cells more or less rounded, and exhibiting small intercellular spaces. Surrounding this is the single clearly defined row of archisporial cells. The sterile amphithecial tissue shows four or five rows of cells outside the archesporium. Higher up the archesporium divides into two layers, and still higher up these divide again more or less completely, so that there are three or four layers of cells (Fig. 54). In *M. Salakensis* the development of the archesporial tissue is somewhat less than in *M. Tjibodensis*, and there are seldom more than three layers of cells in the completed archesporium (Fig. 57).

Soon after the final divisions of the archesporial tissue are complete the differentiation of the sporogenous cells and the elaters begins. There seems to be no definite relation of these to the divisions of the archesporium, such as can be seen in *Notothylas*, and in some species of *Anthoceros*. Certain cells (el) are longer than the other (sp). The latter soon begin to become rounded off and to separate, while the sterile cells rapidly increase in length with the growth of the sporogonium, and push their way between the young spore-mother-cells which thus lie in the meshes of a net-work formed by the coherent elongated sterile cells (Figs. 60, 61). The separation of the spore-mother-cells is due to a disintegration of a part of the original cell-wall, which evidently becomes mucilaginous, and this mucilage stains very strongly, and makes the sporogenous tissue extremely conspicuous in stained sections.

The young spore-mother-cells are nearly round, with a small nucleus which does not stain very strongly. At an early period the single chromatophore can be easily seen. Owing to its small size the divisions of the nucleus are difficult to study, and no attempt was made to follow out the details of the nuclear division. They probably do not differ much from what has been observed in *Anthoceros* (Davis, The Spore Mother Cells of Anthoceros. Bot. Gazette, xxviii, 1899). Davis found four chromosomes in the nucleus of Anthoceros. As in *Anthoceros*, the division of the chromatophore occurs before the nucleus divides. The nucleus of the young cell lies near the centre, more or less enclosed by the cup-shaped chromatophore

(Fig. 63 a). In the next stage (b) the chromatophore divides into two, which assume an oval form, and show clearly a number of starch granules imbedded in them. The two chromatophores are in *Megaceros* connected by a strand of fine fibrils, which Davis does not seem to have seen in *Anthoceros*. About this time there is a marked appearance of synapsis in the nucleus (Fig. 63 b), and Davis says that this is a constant phenomenon, and regards it as entirely normal. The two chromatophores divide again and assume a position marking the tetrad arrangement of the future spores. They are connected, as in the case of the two chromatophores, by strands of fibrils, and have very much the effect of the four nuclei connected by fibrils which are seen in the ordinary type of spore division. The nucleus now divides, as Davis has shown, by successive mitoses, with a resting-stage between, and the four nuclei arrange themselves at the four points occupied by the chromatophores, after which the cell-wall separating the four spores of the tetrad are developed. It was noted that before the division began there was already a slight lobing of the cell, such as is so common in the Jungermanniales, marking the position of the spores. This has also been observed by the writer in *Targionia*.

The outer membrane of the spore becomes somewhat thickened, but remains thinner than is the case in *Anthoceros* and *Notothylas*. In this respect *Megaceros* approaches *Dendroceros*, and the spores agree with those of *Dendroceros* also in the development of chlorophyll, which is wanting in the ripe spores of all typical species of *Anthoceros*.

The elaters, as in *Dendroceros*, are as a rule composed of several cells, and are simply larger or smaller fragments of the net of sterile cells which surrounds the spores. This becomes especially clear in *M. Salakensis*, where the elaters are usually branched (Fig. 72). In *M. Tjibodensis* they are rather more slender and seldom show any branches. The characteristic thickened spiral upon the walls of the elaters first becomes evident about the time that the division of the spores is complete. In both species the ripe spores show fine spinules or papillae upon the outer surface, these being more numerous, finer, and more regular in *M. Salakensis*. The superficial cells of the sporogonium develop thick walls and assume a brownish colour as the sporogonium ripens, but no trace of the stomata characteristic of the typical *Anthoceros* sporogonium is developed. The two species examined differ in the form of the superficial cells of the sporogonium, which are about twice as long in *M. Tjibodensis* as in *M. Salakensis*. In the former species there are two to six chromatophores in each cell of the assimilative tissue of the sporophyte, the commonest number being four.

As the sporophyte develops, rhizoids are formed in great numbers from the lower surface of the thallus below the foot of the sporophyte, the growth of which in *M. Tjibodensis* causes a marked swelling upon the lower

side of the thallus. This is less marked in *M. Salakensis*. In this species also many rhizoids are formed immediately below the sporophyte. These are presumably concerned in supplying the growing sporophyte with water and possibly with food. It would not be surprising if sometimes the rhizoid-like outgrowths of the foot itself might penetrate into the substratum, but no demonstration of this could be made. The spores of both of the species of *Megaceros* under consideration are small—much smaller than in most species of the other genera. The contrast is especially great when compared with the large spores of *Dendroceros* with which *Megaceros* otherwise most nearly agrees in the character of the sporophyte, as well as in the thin walls and green chromatophore of the spores. In both species examined the ripe spore contains a bright green chromatophore and germinates very promptly. In size the spores are about equal in the two species, measuring about 28 μ in diameter. Those of *M. Tjibodensis* (Figs. 66, 69) are less regular in outline than those of *M. Salakensis* (Figs. 73, 75), and the surface papillae are coarser and less uniform in size. The elaters of the two species also differ, as we have seen, those of *M. Tjibodensis* being rather more slender and more seldom branched. They may reach a length of 300 μ, or more, and are usually composed of about three cells, although small unicellular ones (Fig. 68) are sometimes found. In *M. Salakensis* the elaters are almost always markedly branched, and the ends of the branches are often blunt, showing where they have broken away from others, and indicating clearly that the elater is only a part of an extensive net of sterile cells surrounding the spores (Figs. 71, 72).

Germination.

Experiments were made in germinating the spores of *M. Tjibodensis*. The spores germinate promptly, the first signs of germination being usually evident within two days. Within three days the first cell division occurs. Most of the cells of the young plant have a single chromatophore, but occasionally two are found in a cell. No germ tube is found, and the young thallus develops directly from the spore. The first rhizoid is formed earlier than is usually the case in *Anthoceros*.

The Relation of Megaceros to the other Anthocerotaceae.

Megaceros shows points of contact with all three of the other genera. The form of the thallus (at least in the species under consideration) is that of a typical *Anthoceros*, and this is true also of the apical growth which, however, resembles also that of *Notothylas*. The archegonium is perhaps most like that of *Dendroceros*, and this is true also of the large solitary antheridium. The sporophyte resembles most nearly in form and in its large size *Anthoceros*, but in the early divisions of the embryo and the

origin of the sporogenous tissue it is more like *Dendroceros*, with which it agrees also in the green spores, the spiral elaters, and in the absence of stomata. The great development of the sporogenous tissue, as well as the large amount of sporogenous tissue developed in the apical region of the sporophyte, are more like *Notothylas*.

SUMMARY.

1. Probably all of the species of *Anthoceros* in which stomata are absent from the sporogonium, and which have spiral elaters, should be separated as a new genus. The name *Megaceros* is proposed for this.

2. Two species from Java were carefully studied; these appear to be undescribed, and the names *M. Tjibodensis* and *M. Salakensis* are proposed for them; the former is probably closely allied to *M. Stahlii* (Steph.), but seems to be distinct.

3. Both species show multiple chromatophores, and this peculiarity is probably common to all members of the genus. Two tropical American species, *M. Vincentianus* and *M. flavens*, show the same character, and it is also known in *M. giganteus*. In *M. Tjibodensis* as many as twelve chromatophores may be found in a cell. Pyrenoids were absent in all of the forms studied except *M. Salakensis* where there was some evidence that a pyrenoid was present.

4. The form of the thallus and the apical growth are like those of the typical *Anthoceros*.

5. Both of the Javanese species are monoecious, but strongly proterogynous; the archegonia resemble those of *Anthoceros*, but the large solitary antheridium is more like that of *Dendroceros*.

6. The early divisions in the embryo and young sporophyte most nearly resemble *Dendroceros*; the extent of the sporogenous tissue, however, is much greater, and most like *Notothylas*.

7. There is no evident relation between the early divisions of the sporogenous tissue and the distribution of the fertile and sterile cells. The latter form an irregular net enclosing the spore-mother-cells.

8. As in *Anthoceros* the primary chromatophore of the spore-mother-cell divides into four before the nucleus divides; there is a slight lobing of the mother-cell before division takes place; the spores are small, thin-walled, with fine papillae or spinules upon their surface, and contain a large chloroplast.

9. The elaters are multicellular, and closely resemble those of *Dendroceros*; they are branched in *M. Salakensis*, but unbranched in *M. Tjibodensis*.

10. There is a large amount of green tissue developed in the sporophyte, but no stomata are present; in *M. Tjibodensis* the cells of the sporophyte

contain 2–6 chromatophores; the dehiscence of the capsule is either by two valves (*M. Tjibodensis*), or along one suture (*M. Salakensis*).

11. The foot is very large, and the surface cells are developed into extensively branched rhizoid-like outgrowths; rhizoids develop in great numbers from the lower surface of the thallus immediately below the foot.

Megaceros, gen. nov.

Thallus large in all of the Javanese species, closely resembling *Anthoceros*, both in form and in the apical growth; chromatophores several (2–12) in the inner cells, and usually more than one in the superficial ones; pyrenoids are usually absent. Plants (always?) monoecious; antheridia large, solitary; sporophyte large, epidermis without stomata; spores small, thin-walled, papillate or echinulate, at maturity containing a single large chloroplast; elaters either branched or unbranched, multicellular, with a distinct spiral band like that of *Dendroceros*.

Probably all of the species of *Anthoceros* in Gottsche's third section of the genus should be transferred to *Megaceros*.

M. Tjibodensis, sp. nov.

Thallus 2–5 centimetres long; ecostate, 8–12 cells thick in the middle. Margin strongly lobed, and laciniately fringed; monoecious, but strongly proterogynous; antheridia nearly globular, upright, about 135 μ in diameter, short stalked; involucre 10–15 mm. long; sporophyte very long, 6–9 c.; spores 28 μ, somewhat irregular in shape, papillate: elaters long and slender, sometimes 300 μ in length, unbranched; epidermal cells of sporophyte long and narrow. Growing on logs, upon the ground, and upon boulders. Tjibodas; also below Kandang Badak on the slopes of Mount Gedeh, Java; April, May, 1906.

Probably near to *M.* (*Anthoceros*) *Stahlii* (Steph.), but differs in the larger size and greater thickness, the larger spores, and much longer elaters.

M. Salakensis, sp. nov.

Thallus smaller than *M. Tjibodensis*, about 2–3 centimetres long, 8–10 cells thick in the middle, crenately lobed but not laciniate; chromatophores 1–2 in the superficial cells, 2–6 in the inner ones, a pyrenoid probably present in the larger ones; monoecious, proterogynous; antheridia oval, about 180 μ in length exclusive of the curved pedicel which is much longer than in *M. Tjibodensis*, and attached at the forward part of the antheridial chamber; sporophyte much shorter than in *M. Tjibodensis*, 3–4 centimetres long; spores 28 μ, nearly round, and finely echinulate; elaters branched, somewhat thicker than in *M. Tjibodensis*, epidermal cells of the sporophyte about half as long as in *M. Tjibodensis*. On a dead log, among mosses; Tjiapus Gorge, foot of Mount Salak, Java; March, 1906.

EXPLANATION OF PLATES XLIV–XLVI.

Illustrating Professor Campbell's paper on Anthocerotaceae.

PLATE XLIV.

Fig. 1. A rather small plant of the typical *Megaceros Tjibodensis*, with two young sporophytes, *sp.* × 2.

Fig. 2. An older sporophyte, showing the long involucre, *in.* × 2.

Fig. 3. A ripe sporophyte removed from the involucre. Natural size.

Fig. 4. A plant of *M. Salakensis*, with two young sporophytes. × 2.

Fig. 5. Ripe sporophyte of *M. Salakensis.* × 2.

Fig. 6. Margin of the thallus of the typical *M. Tjibodensis.* × 25.

Fig. 7. Margin of the thallus of a larger form of the same. × 25.

Fig. 8. Margin of thallus of *M. Salakensis.* × 25.

Fig. 9. Cells from upper surface of *M. Tjibodensis*, showing the chromatophores. × 280.

Fig. 10. Cells from the lower surface of the same species.

Fig. 11. Cell from the interior of the thallus of *M. Tjibodensis*, showing twelve chromatophores. × 280.

Fig. 12. Cell from the interior of the thallus of a form growing with *M. Tjibodensis*, but showing a different type of chromatophore. × 280.

Figs. 13–17. Chromatophores of *M. Salakensis.* × 280. Fig. 13, cells from upper surface; 14, from lower surface; 15, a sub-epidermal cell; 16, 17, inner cells. *n*, the nucleus.

Fig. 18. Cell from the interior of the thallus of *M. flavens* (Spruce). × about 500.

Fig. 19. Cell from the interior of the thallus of *M. Vincentianus* (Lehm. and Lindenb.). × about 500.

Fig. 20. Longitudinal section of the apex of the thallus of *M. Tjibodensis.* × 280. ♀, a young archegonium.

Fig. 21. Apical region of *M. Tjibodensis*, showing an approach to the type of *Dendroceros.*

Fig. 22. Longitudinal section of the thallus of *M. Tjibodensis*, showing the relative position of the antheridium, ♂, and the archegonium, ♀; the latter contains a young embryo; *N*, Nostoc colony. × 25.

Figs. 23–5. Development of the young antheridium of *M. Salakensis*, seen in longitudinal section. × 480. *b*, the basal cell.

Fig. 26. The outer cells of the antheridium shown in Fig. 25.

Fig. 27. Young antheridium of *M. Salakensis* in which the antheridial chamber is open on one side. × 480.

Fig. 28. An older stage of the same species. × 280.

Fig. 29. Two sections of a nearly ripe antheridium of *M. Salakensis.* × 280.

Fig. 30. Nearly ripe spermatocytes. × about 900.

PLATE XLV.

Fig. 31. Median section of a nearly ripe antheridium of *M. Tjibodensis.* × 280.

Fig. 32. Cross-section of the pedicel of the antheridium.

Figs. 33, 34. Two very young archegonia of *M. Tjibodensis*, longitudinal sections. × about 600. In Fig. 34 the outer cell had divided by a longitudinal wall before the separation of the primary neck canal cell had taken place.

Figs. 35, 36. Two older stages of the archegonium. × 480. In the one shown in 36 there were four cover cells.

Fig. 37. A nearly ripe archegonium of the same species. × 280.

Fig. 38. Cross-section of the venter of a young archegonium. × 480.

Fig. 39. Two cross-sections of the upper part of the neck of a nearly ripe archegonium. × 280. Three cover cells, *d*, are present.

Fig. 40. A mature archegonium of *M. Salakensis.* × 280.

Fig. 41. Longitudinal section of a fertilized archegonium of *M. Tjibodensis*, containing an eight-celled embryo. × 280.

Fig. 42. An older embryo of the same species. × 285. *I-I*, the primary wall; *II-II*, the second wall.

Figs. 43, 44. Median sections of older embryos of *M. Tjibodensis*. × 280. The nuclei of the archesporial cells are shown.

Figs. 45–7. A series of four transverse sections of an embryo of *M. Tjibodensis*, of about the same age as the one shown in Fig. 44; *a* is the apex of the embryo.

Fig. 48. Longitudinal section of a fertilized archegonium of *M. Salakensis*, containing a four-celled embryo. × 280.

Fig. 49. An older embryo of *M. Salakensis*. × 280.

Fig. 50. Two cross-sections of a very young embryo of *M. Salakensis*. × 280. The divisions in the lower section, *b*, are very irregular.

Fig. 51. Series of three transverse sections of an embryo of *M. Salakensis*. × 280. The primary wall, *I*, is indicated in the upper section, *a*.

Fig. 52. Median section of a young sporophyte of *M. Tjibodensis*. × 110. The archesporial tissue is shaded.

Fig. 53. The upper part of Fig. 52. × 280.

Fig. 54. Part of a median longitudinal section of an older sporophyte of *M. Tjibodensis*, showing the extent of the archesporial tissue, *sp*; the columella, *col*; and the wall-tissues, *w*. × 280.

Fig. 55. Median section of young sporophyte of *M. Salakensis*. × 110.

Fig. 56. Upper part of Fig. 55. × 280.

Fig. 57. Part of median section of older sporophyte of *M. Salakensis*. × 280.

PLATE XLVI.

Fig. 58. Cross-section of the sporophyte of *M. Salakensis* at a point where the archesporium consists of a single layer of cells; the nuclei are shown in the archesporial cells. × 280.

Fig. 59. Archesporium of *M. Tjibodensis*, showing the first differentiation of the sterile cells, *el*, and the spore-mother-cells, *sp*. × 280.

Figs. 60, 61. More advanced stages in the segregation of fertile and sterile cells; Fig. 61 is a tangential section. × 280.

Fig. 62. Section of the sporophyte of *M. Tjibodensis*, showing nearly ripe spore-tetrads, *sp*, and elaters, *el*. × 280. *col*, columella; *w*, wall-cells.

Fig. 63. Spore-mother-cells of *M. Tjibodensis*, showing the division of the chromatophores. × about 900.

Fig. 64. First nuclear division in spore-mother-cell; the cell is slightly lobed, indicating the position of the spores.

Fig. 65. Two ripe spores of *M. Tjibodensis*. × 280.

Fig. 66. A single spore, more highly magnified.

Figs. 67, 68. Elaters of *M. Tjibodensis*. × 280.

Fig. 69. Section of a ripe spore of *M. Tjibodensis*. × about 900.

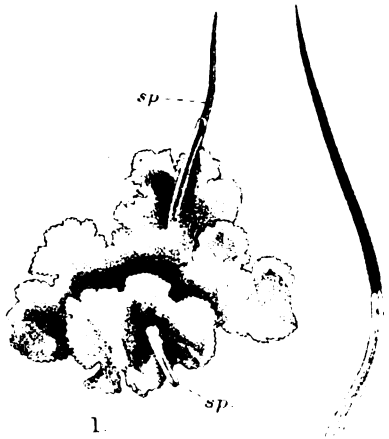
Fig. 70. Part of the surface of the spore.

Figs. 71, 72. Elaters of *M. Salakensis*. × 280.

Fig. 73. Two ripe spores of *M. Salakensis*. × 280.

Fig. 74. Spore of the same, more highly magnified.

Fig. 75. Sections of ripe spores. × about 900. *b* shows the surface sculpturing.



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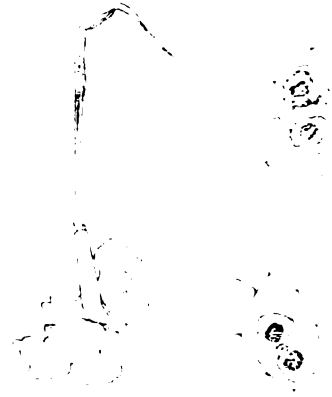

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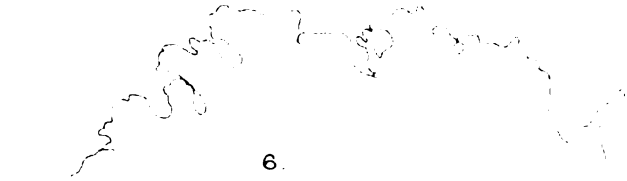

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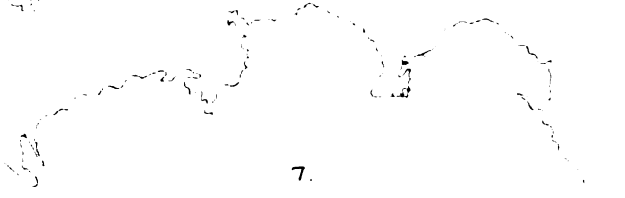

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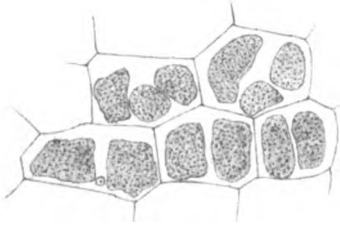

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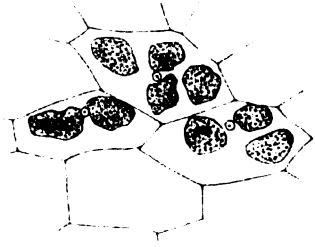

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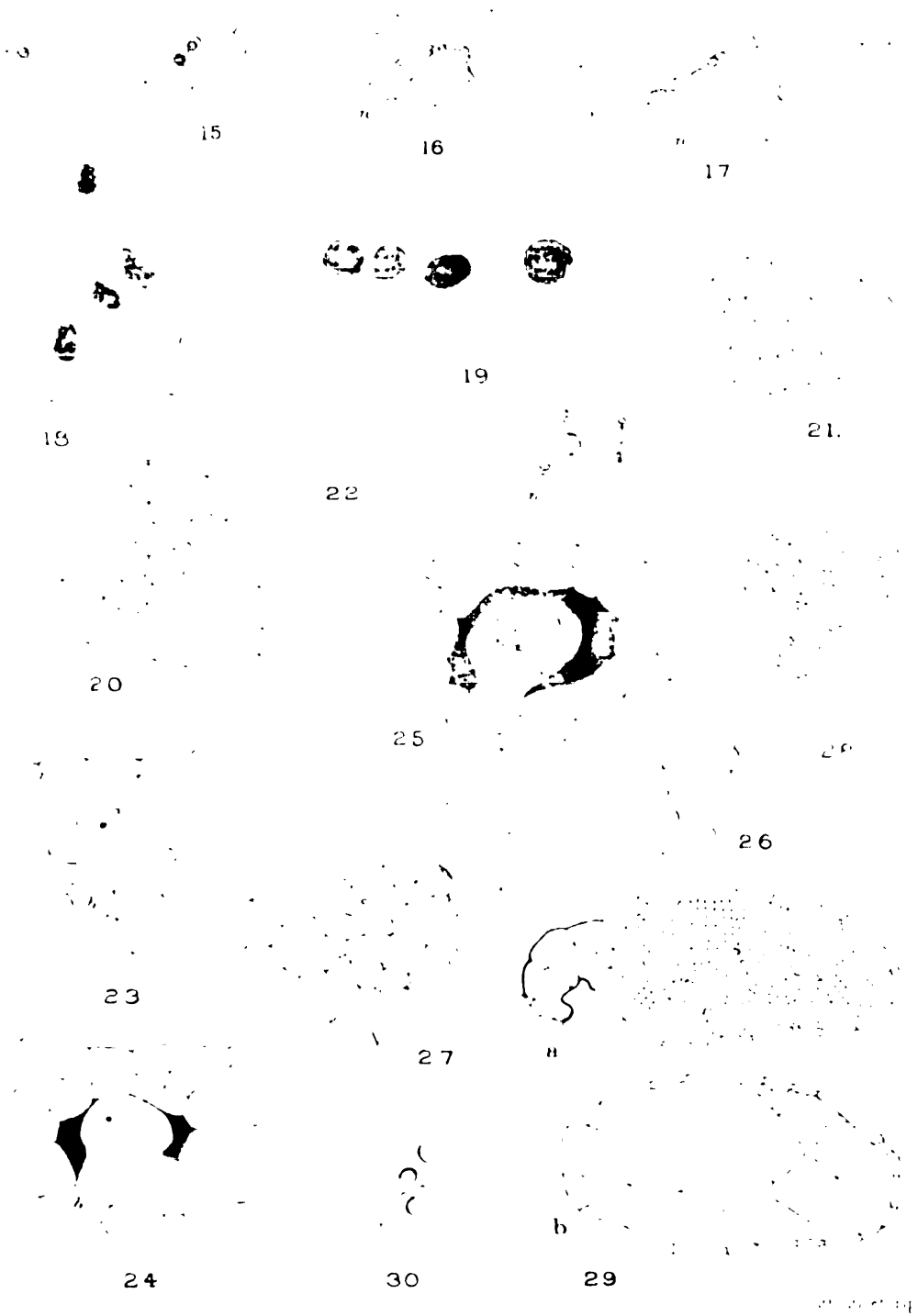
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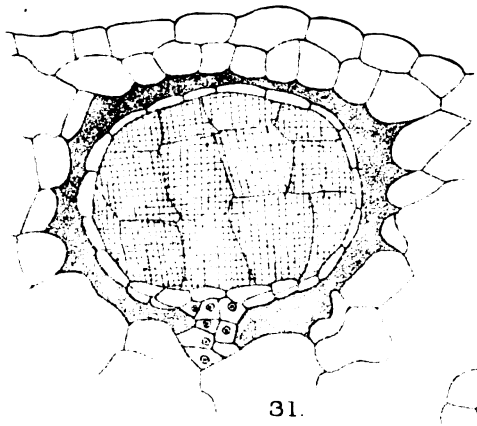

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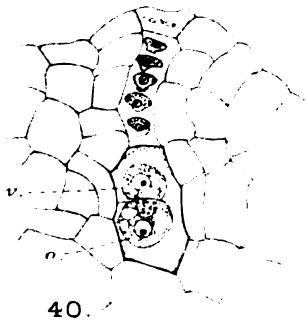

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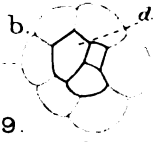

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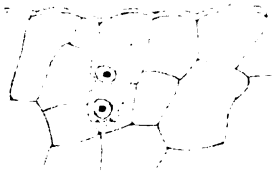

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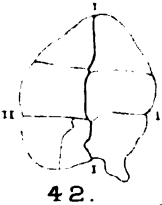

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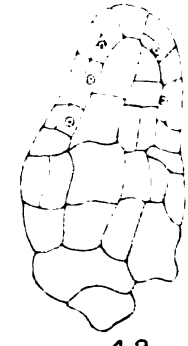

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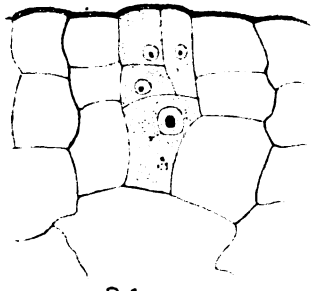

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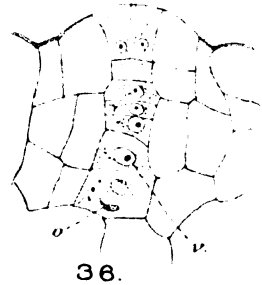

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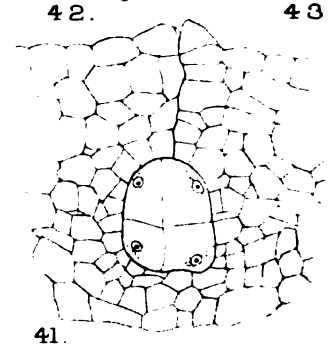

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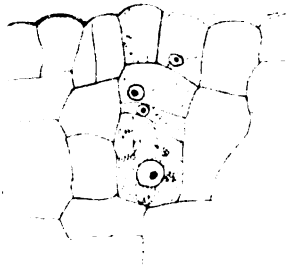

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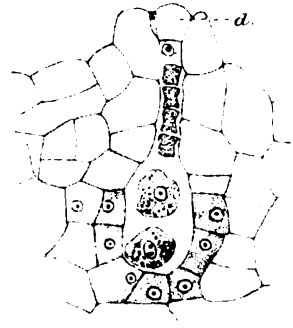

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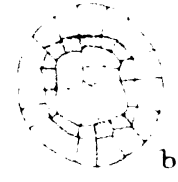

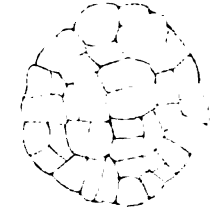

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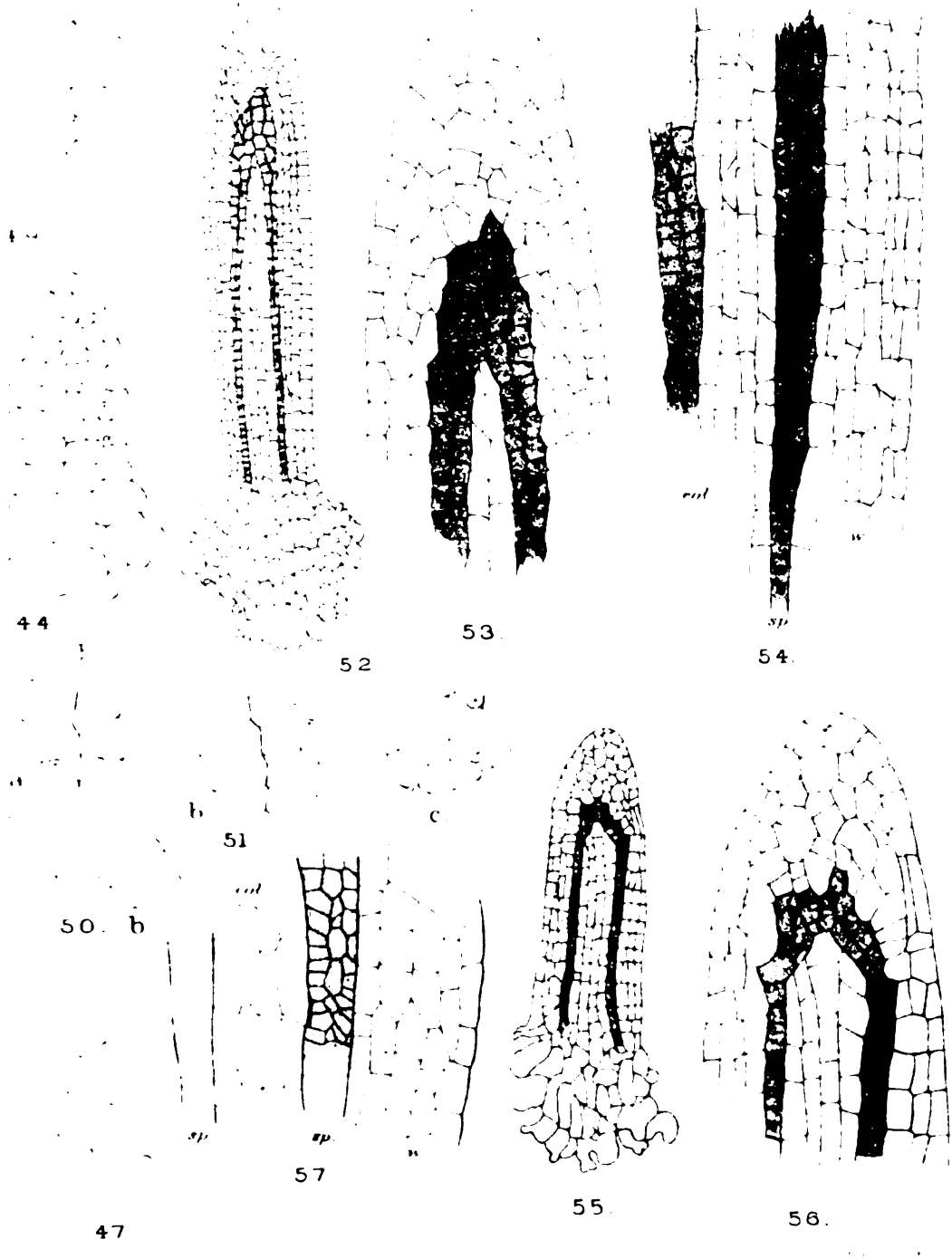
CAMPBELL.—ANTHOCEROTACEAE.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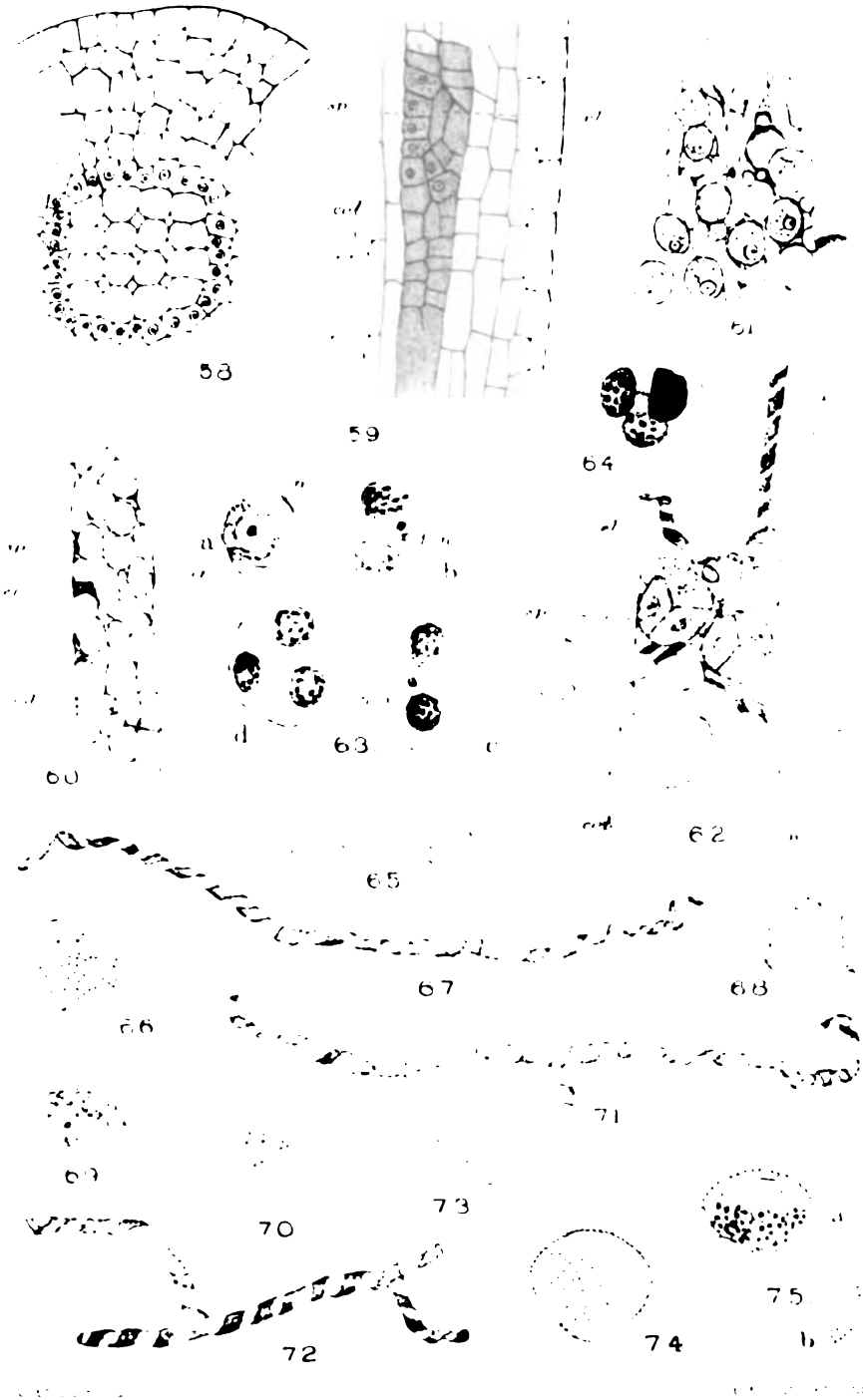

CAMPBELL — ANTHOCEROTACEAE

The Influence of Pollination on the Respiratory Activity of the Gynaeceum.

BY

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THE fact has now been known for some time that injuries and also small doses of poisonous substances produce a kind of feverish reaction on the part of the plant, resulting in a resumption or increased rapidity of growth, accompanied by an increase in the respiratory activity and in the production of heat[1]. The two latter phenomena necessarily go together, but an increase in the rate of growth need not always be accompanied by a rise in the relative respiratory activity, as, for instance, when a stem seems to grow more rapidly simply because the apical internodes retain the power of elongating for a longer time than usual. Conversely, in the case of an injured potato or onion, as shown by Richards[2], the amount of growth is altogether disproportionate to the rise in the respiratory activity, and, in fact, in the case of the onion, little or no growth takes place after injury.

It is evident, therefore, that when a gynaeceum begins its second period of growth, as the result of pollination, the respiratory activity might either remain unaltered, rise, or even conceivably fall as the result of more active production of adult, feebly respiring cells. The latter ultimately takes place as the period of complete ripeness is reached, and this fall is slowly continued until the fruit and seed become decayed or completely dried. No exact comparative determinations appear ever to have been made of the relative respiratory activities of pollinated and unpollinated gynaeceums in as young a condition as possible, so that a reasonable comparison can be made. Naturally, if the flower is one of short duration, and the experiments are delayed, the unpollinated gynaeceum could only be expected to exhibit a lower respiratory activity, but, in the following investigation, care was taken to carry out the experiments while the flowers were quite young, and to determine the activity of respiration while the unpollinated gynaeceum was still active and receptive.

[1] The literature up to 1902–1906 is given in Pfeffer's Physiology of Plants, the English translation.

[2] Annals of Botany, vol. x, No. XL, December, 1896.

[Annals of Botany, Vol. XXI. No. LXXXIV. October, 1907.]

Flowers were used from as widely separated orders as possible, so as to test the range of variation. Owing to the difficulty of preventing the pollination of the native Australian types growing in the open bush under their natural conditions, it was not possible to do much work with them.

The apparatus used throughout the experiments was Aubert's improved form of that of MM. G. Bonnier and L. Mangin for gas analysis. This apparatus has been described in full by Richards in his paper mentioned before.

I found that, after becoming accustomed to this instrument by trying a few preliminary experiments, it worked with perfect satisfaction, provided that the mercury was pure and clean, and that the sodium hydrate and pyrogallol were used in the proper concentration. The complaints from certain quarters as to the lack of accuracy of the apparatus are due to inattention to these precautions.

Method of Experimenting.

The anthers were removed from some of the flower buds while quite young and undehisced, and while the pistil was quite immature.

In many cases this had to be done in extremely young buds, owing to the flowers being strongly protandrous, the anthers depositing their pollen on the still immature stigma, which pollen adhered, and would have served for autogamous fertilization even if access of pollen from other sources were cut off.

The flowers from which the anthers had been removed were tied up securely in loose muslin bags, while other buds, generally on the same plant, of similar age were marked.

These latter were watched, and directly the stigma was seen to be receptive were pollinated with the pollen from other flowers, either on the same or adjacent plants. These flowers were then left for a period extending over a day and a half to six days, the length of the period depending on such conditions as temperature, atmospheric moisture, and the length of the style in those particular flowers.

At the end of this period a certain number of the pollinated and unpollinated flowers were removed, and their respiratory activity tested. Usually they were tested at once, in other cases they were kept with their stems in water for several hours before they were used. The peduncle, sepals, petals, and stamens or filaments were removed very carefully in order to prevent, as much as possible, any stimulating effect on the respiratory activity which might be caused by injury. Since the experiments usually were completed during the latent period of the wound-reaction, and since the gynaeceums were similarly treated in both cases, the disturbing action of this factor can be reasonably neglected.

As a rule the complete gynaeceum was used, but in the case of flowers

having inferior ovaries, such as the *Fuchsia*, it was impossible to retain the style and stigma throughout the experiment, so that the ovaries alone were taken.

The gynaeceums or ovaries were then weighed, the experiments being performed in pairs, pollinated and unpollinated alternately, the weights of material employed in each pair of experiments being as nearly equal as possible.

The gynaeceums were put into a glass tube graduated into cubic centimetres over mercury, and an almost imperceptible quantity of water was passed up to the air space containing the gynaeceums, to avoid any deleterious effects which might be produced by the mercury vapour.

The tubes were placed upright with their open ends immersed in a dish of mercury and left for a certain time, during which they were covered with black cloth to prevent any complications due to carbon-dioxide assimilation. The gynaeceums were necessarily placed in a small volume of air in order to obtain sufficient carbon-dioxide for analysis in a short time, the general time during which they were left in the tubes being two hours.

The detailed results of the experiments are given below in tabulated form. In every case the weight of carbon-dioxide produced was calculated from the volume after correction to normal pressure and temperature.

GYNAECEUMS OF *EUCALYPTUS CALOPHYLLA*, R. BROWN

Weight of material in milligrams.	*Date.*	*Condition of material.*	*Temperature.*	*Vol. of air enclosed.*	*Vol. per cent. of O_2 absorbed by material.*	*Milligrams of CO_2 produced by 10 grms. of material per hour.*	*Duration of experiment.*	*Resp. quotient $\frac{CO_2}{O_2}$ volume.*
625	7/3/07	Pollinated flowers opened 4 days	19·3° C.	5 c.c.	14·1	2·5	2 hours	·62
625	7/3/07	Unpollinated flowers opened 4 days	19·3° C.	5 c.c.	17·5	1·6	2 hours	·33
675	7/3/07	Pollinated flowers opened 4 days	19·3° C.	5 c.c.	16·5	2·4	2 hours	·52
705	7/3/07	Unpollinated flowers opened 4 days	19·3° C.	5 c.c.	13·2	1·6	2 hours	·37
775	8/3/07	Pollinated flowers opened 5 days	18·2° C.	5 c.c.	13·4	1·8	2 hours	·38
665	8/3/07	Unpollinated flowers opened 5 days	18·2° C.	5 c.c.	9·0	1·0	2 hours	·39

The high percentage of oxygen absorbed by the pistils of *Eucalyptus*

calophylla is an interesting phenomenon, in all cases the percentage of oxygen absorbed being greatly in excess of the carbon-dioxide produced. This is probably due to the oily nature of the ovary, some of the oxygen combining with the hydrogen of the hydrocarbon to form water, and only that which combines with the carbon appearing as an equal volume of gaseous carbon-dioxide.

The ratios between the respiratory activities of the pollinated and unpollinated gynaeceums average respectively sixty-seven and forty-two, whilst the respiratory quotient is higher in the pollinated material four days after pollination, but appears to fall to about the same level on the fifth, although the difference in the respiratory activities is as pronounced as on the fourth day.

GYNAECEUMS OF *FUCHSIA SERRATIFOLIA*, RUIZ ET PAVON

Weight of material in milligrams.	*Date.*	*Condition of material.*	*Temperature.*	*Vol. of air enclosed.*	*Vol. per cent. of O_2 absorbed by material.*	*Milligrams of CO_2 produced by 10 grms. of material per hour.*	*Duration of experiment.*	*Resp. quotient $\frac{CO_2 \text{ volume.}}{O_2}$*
300	19/11/06	Pollinated flowers opened 4 days	18° C.	5 c.c.	3·4	2·4	2·3 hours	·53
250	19/11/06	Unpollinated flowers opened 4 days	18° C.	5 c.c.	1·7	1·5	1·8 hours	·43
270	21/11/06	Pollinated flowers opened 5 days	19° C.	5 c.c.	3·6	4·0	2 hours	·69
290	21/11/06	Unpollinated flowers opened 5 days	19° C.	5 c.c.	3·1	2·2	2 hours	·45
480	26/11/06	Pollinated flowers opened 5 days	17·5° C.	5 c.c.	4·0	2·1	2 hours	·57
400	26/11/06	Unpollinated flowers opened 5 days	17·5° C.	5 c.c.	3·6	1·6	2 hours	·39
455	1/12/06	Pollinated flowers opened 4 days	18·5° C.	5 c.c.	1·9	2·7	2·3 hours	·79
265	1/12/06	Unpollinated flowers opened 4 days	18·5° C.	5 c.c.	3·9	1·6	2 hours	·50

In *Fuchsia* the weight of carbon-dioxide expired per 10 grms. of material was fairly constant, except in one pair of experiments which were performed on a different day from the other pairs. For no apparent reason the weight of carbon-dioxide given off in both the pollinated and unpollinated material was nearly double the amount evolved on the preceding and subsequent days. In many instances, throughout the experiments,

similar irregularities occurred, due in all probability to local variations or to nutritional factors. The position of the flower on the plant, whether it is terminal or lateral at the base or apex, and whether it is shaded or exposed, are all factors which influence the vigour of flowers, and hence also their respiratory activities.

The average ratios for the respiratory activity of the pollinated and unpollinated material are in this case as 112 : 69. The respiratory quotient is less than unity, and is throughout higher in the pollinated than in the unpollinated gynaeceums, the average difference being practically the same on both the fourth and fifth days.

GYNAECEUMS OF *PELARGONIUM ZONALE*, L'HÉRIT.

Weight of material in milligrams.	*Date.*	*Condition of material.*	*Temperature.*	*Vol. of air enclosed.*	*Vol. per cent. of O_2 absorbed by material.*	*Milligrams of CO_2 produced by 10 grms. of material per hour.*	*Duration of experiment.*	*Resp. quotient $\frac{CO_2 \text{ volume.}}{O_2}$*
30	25. 3 07	Pollinated flowers opened 5 days	18° C.	3 c.c.	2·7	38·0	2 hours	·92
25	25 3 07	Unpollinated flowers opened 5 days	18° C.	3 c.c.	2·0	4·9	2 hours	·75
40	3 4 07	Pollinated flowers opened 6 days	17° C.	3·5 c.c.	1·6	22·0	2 hours	1·2
20	3 4 07	Unpollinated flowers opened 6 days	17 C.	3·5 c.c.	1·9	4·6	2 hours	·1
30	3/4 07	Pollinated flowers opened 6 days	17° C.	3·5 c.c.	2·9	35·0	2 hours	1·2
20	3 4 07	Unpollinated flowers opened 6 days	17° C.	3·5 c.c.	2·4	6·8	2 hours	·28

Conspicuous irregularities are noticeable in the respiratory quotient of *Pelargonium zonale*. These were caused mainly by the extremely large differences between the CO_2 production in the pollinated and unpollinated material, the differences between the O_2 absorption being less pronounced.

The average relative respiratory activities were :—pollinated, 950 ; unpollinated, 163 ; the difference being more pronounced on the fifth day than on the sixth day. The respiratory quotient on the fifth day was nearly the same in both lots of material, but on the sixth day rose above unity in the case of the pollinated, and fell to quite a small fraction in the case of the unpollinated material. Possibly the stimulus of pollination hastens or induces the passage of a certain amount of oily food material to the gynaeceum.

GYNAECEUMS OF *DIGITALIS PURPUREA*, L.

Weight of material in milligrams.	*Date.*	*Condition of material.*	*Temperature.*	*Vol. of air enclosed.*	*Vol. per cent. of O_2 absorbed by material.*	*Milligrams of CO_2 produced by 10 grms. of material per hour.*	*Duration of experiment.*	*Resp. quotient $\frac{CO_2}{O_2}$ volume.*
295	5/12/06	Pollinated flowers opened 5 days	17° C.	5 c.c.	6·7	6·9	2 hours	·65
290	5/12/06	Unpollinated flowers opened 5 days	17° C.	5 c.c.	5·0	3·2	2·2 hours	·44
295	10/12/06	Pollinated flowers opened 5 days	20° C.	5 c.c.	11·2	15·0	2 hours	·87
255	10/12/06	Unpollinated flowers opened 5 days	20° C.	5 c.c.	9·9	10·9	2 hours	·61
260	12/12/06	Pollinated flowers opened 5 days	17·5° C.	5 c.c.	4·3	8·8	2 hours	·74
265	12/12/06	Unpollinated flowers opened 5 days	17·5° C.	5 c.c.	3·9	5·5	2 hours	·56

As in previous cases, if the production of CO_2 is taken as representing the respiratory activity, the pollinated material produces 307 grams of CO_2 in the same time as a similar weight of unpollinated material produces 196 grams. There is, however, a wide range between the means of successive pairs of experiments. The respiratory quotients for the pollinated material average 0·75, and for the unpollinated 0·54, showing less fluctuation and a smaller average difference than in any previous case.

GYNAECEUMS OF *BEGONIA SEMPERFLORENS*, LINK

Weight of material in milligrams.	*Date.*	*Condition of material.*	*Temperature.*	*Vol. of air enclosed.*	*Vol. per cent. of O_2 absorbed by material.*	*Milligrams of CO_2 produced by 10 grms. of material per hour.*	*Duration of experiment.*	*Resp. quotient $\frac{CO_2}{O_2}$ volume.*
300	11/4/07	Pollinated flowers opened 4 days	15° C.	5 c.c.	1·9	2·1	2 hours	·73
380	11/4/07	Unpollinated flowers opened 4 days	15° C.	5 c.c.	2·7	·4	2 hours	·29
265	12/4/07	Pollinated flowers opened 5 days	16° C.	5 c.c.	1·9	2·4	2·1 hours	·73
325	12/4/07	Unpollinated flowers opened 5 days	16° C.	5 c.c.	1·6	·7	2·2 hours	·32

In the case of *Begonia semperflorens* 45 milligrams of carbon-dioxide were produced by the pollinated gynaeceums, as compared with 11 milligrams obtained from an equal weight of the unpollinated.

The average respiratory quotients of the pollinated specimens and unpollinated specimens, respectively, are as ·73 : ·31.

GYNAECEUMS OF *TECOMA CAPENSIS*, R. BROWN

Weight of material in milligrams.	*Date.*	*Condition of material.*	*Temperature.*	*Vol. of air enclosed.*	*Vol. per cent. of O_2 absorbed by material.*	*Milligrams of CO_2 produced by 10 grms. of material per hour.*	*Duration of Experiment.*	*Resp. quotient $\frac{CO_2}{O_2}$ volume.*
50	27/3/07	Pollinated flowers opened 5 days	12·5° C.	6 c.c.	3·0	30·0	2 hours	·53
60	27/3/07	Unpollinated flowers opened 5 days	12·5° C.	6 c.c	2·9	15·0	2 hours	·34
60	27/3/07	Pollinated flowers opened 5 days	12·5° C.	5 c.c.	1·9	7·0	2 hours	·36
60	27/3/07	Unpollinated flowers opened 5 days	12·5° C.	4 c.c.	1·9	3·1	2 hours	·11
60	4/4/07	Pollinated flowers opened 5 days	16·5° C	5 c.c.	1·3	4·6	2 hours	·35
65	4/4/07	Unpollinated flowers opened 5 days	16·5° C.	5 c.c.	1·0	2·8	2 hours	·28

In *Tecoma capensis*, out of two pairs of experiments performed on the same day there was a pronounced difference in the carbon-dioxide production between the first and second pair. The fall in the oxygen absorption was not so marked, but it is evident that the respiratory activity rapidly decreased in the flowers cut and kept with their cut ends under water. Whether this was due to a closure of stomata, or to the consumption of the presumably limited supply of food material available, is difficult to say. In any case the respiratory activities of the pollinated and unpollinated gynaeceums are as 416 : 209, and the average respiratory quotients as 0·41 : 0·24.

GYNAECEUMS OF *TROPAEOLUM MAJUS*, L.

Weight of material in milligrams.	*Date.*	*Condition of material.*	*Temperature.*	*Vol. of air enclosed.*	*Vol. per cent. of O_2 absorbed by material.*	*Milligrams of CO_2 produced by 10 grms. of material per hour.*	*Duration of experiment.*	*Resp. quotient $\frac{CO_2}{O_2}$ volume.*
40	3/12/06	Pollinated flowers opened about 6 days	16° C.	2 c c.	3·6	11·7	2 hours	·66
30	3/12/06	Unpollinated flowers opened 6 days	16° C.	2 c.c.	3·5	10·1	2·2 hours	·51
45	8/12/06	Pollinated flowers opened 6 days	20° C.	2 c.c.	4·5	12·0	2 hours	·68
35	8/12/06	Unpollinated flowers opened 6 days	20° C.	2 c.c.	2·8	4·4	2 hours	·30
45	8/12/06	Pollinated flowers opened 6 days	20° C.	2 c.c.	3·3	11·0	2 hours	·75
35	8/12/06	Unpollinated flowers opened 6 days	20° C.	2 c.c.	3·9	8·8	2 hours	·43

Comparing the pollinated with the unpollinated material as before, 100 grams of the former were found to produce 347 milligrams of carbon-dioxide, while that produced by 100 grams of the latter was 233 milligrams. The respiratory quotients of the pollinated material are fairly constant, as are also those of the unpollinated, the average quotient in the first case being 0·69, and in the second case being 0·41.

GYNAECEUMS OF *CHEIRANTHUS CHEIRI*, L.

Weight of material in milligrams.	*Date.*	*Condition of material.*	*Temperature.*	*Vol. of air enclosed.*	*Vol. per cent. of O_2 absorbed by material.*	*Milligrams of CO_2 produced by 10 grms. of material per hour.*	*Duration of experiment.*	*Resp. quotient $\frac{CO_2}{O_2}$ volume.*
100	11/10/06	Pollinated flowers opened 5 days	11° C.	5 c.c.	4·6	15·0	2 hours	·69
100	11/10/06	Unpollinated flowers opened 5 days	11° C.	5 c.c.	2·6	5·3	2 hours	·46
100	24/10/06	Pollinated flowers opened 4 days	12° C.	5 c.c.	4·5	16·0	2·3 hours	·53
100	24/10/06	Unpollinated flowers opened 4 days	12° C.	5 c.c.	4·0	10·0	2 hours	·57

Irregularities manifest themselves in the previous set of experiments, though the data show a higher degree of respiratory activity in the pollinated specimens than in the unpollinated, as represented by the weight of carbon-dioxide evolved. The difference between the specimens which had been pollinated for five days was twenty, whilst in those which had been pollinated four days it was five.

All through the experiments it was a practically universal rule that the respiratory quotient was higher in the pollinated gynaeceums, but here an exception is observed in the second pair of experiments.

GYNAECEUMS OF *ANTIRRHINUM MAJUS*, L.

Weight of material in milligrams.	*Date.*	*Condition of material.*	*Temperature.*	*Vol. of air enclosed.*	*Vol. per cent. of O_2 absorbed by material.*	*Milligrams of CO_2 produced by 10 grms. of material per hour.*	*Duration of experiment.*	*Resp. quotient $\frac{CO_2}{O_2}$ volume.*
48	7/12/06	Pollinated, corolla just falling off, opened 5 days	21° C.	4 c.c.	3·4	9·5	2·5 hours	·56
48	7/12/06	Unpollinated flowers opened the same time as above	21° C.	4 c.c.	1·3	4·5	2·5 hours	·69
135	8/4/07	Pollinated flowers opened 5 days	17·5° C.	5 c.c.	3·0	7·5	2 hours	·73
120	8/4/07	Unpollinated flowers opened 5 days	17·5° C.	5 c.c.	2·2	1·5	2 hours	·18
120	8/4/07	Pollinated flowers opened 5 days	17·5° C.	5 c.c.	4·0	12·0	2·3 hours	·95
120	8/4/07	Unpollinated flowers opened 5 days	17·5° C.	5 c.c.	3·0	5·0	2·3 hours	·50

The carbon-dioxide production in the pollinated material bears a ratio to the production in the unpollinated material of 290 : 110.

Also the average respiratory quotients were as 0·75 : 0·46.

GYNAECEUMS OF *ANEMONE JAPONICA* VAR. *ALBA*, SIEB. ET ZUCC.

Weight of material in milligrams.	*Date.*	*Condition of material.*	*Temperature.*	*Vol. of air enclosed.*	*Vol. per cent. of O_2 absorbed by material.*	*Milligrams of CO_2 produced by 10 grms. of material per hour.*	*Duration of experiment.*	*Resp. quotient $\frac{CO_2}{O_2}$ volume.*
650	5/4/07	Pollinated flowers opened 5 days	18·5° C.	5 c.c.	5·2	·28	2 hours	·69
480	5/4/07	Unpollinated flowers opened 5 days	18·5° C.	5 c.c.	6·1	·28	2 hours	·49
380	8/4/07	Pollinated flowers opened 5 days	18·5° C.	5 c.c.	5·2	·51	2 hours	·82
390	8/4/07	Unpollinated flowers opened 5 days	18·5° C.	5 c.c.	6·4	·51	2 hours	·68

Practically the same results were obtained from the pollinated and unpollinated specimens of *Anemone japonica*.

As no trace of fruit formation could be found in any one flower out of an extremely large number which were examined and watched, it was evident that the pollen was infertile, and that all the specimens used were really unpollinated. The experiments serve to show, however, that in similar pairs of gynaeceums the production of carbon-dioxide is very constant, although the respiratory quotient fluctuates quite appreciably. From the variation in the respiratory quotient it can be concluded that the variations shown in the case of *Cheiranthus Cheiri* are also independent of pollination.

GYNAECEUMS OF *LILIUM CANDIDUM*, L.

Weight of material in milligrams.	*Date.*	*Condition of material.*	*Temperature.*	*Vol. of air enclosed.*	*Vol. per cent. of O_2 absorbed by material.*	*Milligrams of CO_2 produced by 10 grms. of material per hour.*	*Duration of experiment.*	*Resp. quotient $\frac{CO_2}{O_2}$ volume.*
340	12/12/06	Pollinated flowers opened 5 days	16·5° C.	15 c.c.	5·4	2·0	16 hours	·68
515	12/12/06	Unpollinated flowers opened 5 days	16·5° C.	15 c.c.	6·1	1·2	16 hours	·60

In experimenting with Monocotyledons, irregularities in both CO_2 production and O_2 absorption were found to be of more frequent occurrence than in Dicotyledons.

Also the respiratory activity of the gynaeceum was in each case much feebler than in the Dicotyledons.

From the data given for the experiments on *Lilium candidum* it will be seen that it was necessary to leave the material enclosed in the tube for many hours before any appreciable change in the enclosed air was observed.

GYNAECEUMS OF *CANNA INDICA*, L.

Weight of material in milligrams.	*Date.*	*Condition of material.*	*Temperature.*	*Vol. of air enclosed.*	*Vol. per cent. of O_2 absorbed by material.*	*Milligrams of CO_2 produced by 10 grms. of material per hour.*	*Duration of experiment.*	*Resp. quotient $\frac{CO_2 \text{ volume.}}{O_2}$*
290	13/3/07	Pollinated flowers opened 7 days	19·6° C.	5 c.c.	1·9	2·0	1·7 hours	·57
205	13/3/07	Unpollinated flowers opened 7 days	19·6° C.	5 c.c.	3·2	·5	1·7 hours	·62
270	20/3/07	Pollinated flowers opened 5 days	16·5° C.	5 c.c.	2·7	1·6	2 hours	·36
320	20/3/07	Unpollinated flowers opened 5 days	16·5° C.	5 c.c.	3·0	·1	2 hours	·03
240	20/3/07	Pollinated flowers opened 5 days	16·5° C.	5 c.c.	3·2	3·9	2·2 hours	·72
300	20/3/07	Unpollinated flowers opened 5 days	16·5° C.	5 c.c.	2·4	·6	2·2 hours	·20

The principal fact noted here was that the carbon-dioxide given off was markedly less than in the experiments previously given on other flowers.

The ratio of the carbon-dioxide evolved by the pollinated to that evolved by the unpollinated specimens was $\frac{75}{12}$.

The respiratory quotient of the former was to that of the latter as 0·55 is to 0·28.

GYNAECEUMS OF *AGAPANTHUS UMBELLATUS* VAR. *ALBIFLORUS*, L'HÉRIT.

Weight of material in milligrams.	*Date.*	*Condition of material.*	*Temperature.*	*Vol. of air enclosed.*	*Vol. per cent. of O_2 absorbed by material.*	*Milligrams of CO_2 produced by 10 grms. of material per hour.*	*Duration of experiment.*	*Resp. quotient $\frac{CO_2}{O_2}$ volume.*
200	16/3/07	Pollinated flowers opened 6 days	19·6° C.	5 c.c.	1·9	2·5	2 hours	·57
190	16/3/07	Unpollinated flowers opened 6 days	19·6° C.	5 c.c.	1·7	2·3	2 hours	·59
235	16/3/07	Pollinated flowers opened 6 days	19·6° C.	5 c.c.	3·3	2·3	2 hours	·36
220	16/3/07	Unpollinated flowers opened 6 days	19·6° C.	5 c.c.	2·7	1·6	2 hours	·29
240	22/3/07	Pollinated flowers opened 7 days	18° C.	5 c.c.	3·1	1·5	2 hours	·17
240	22/3/07	Unpollinated flowers opened 7 days	18° C.	5 c.c.	3·3	·19	2 hours	·63

Except in one pair of experiments in *Agapanthus*, in which the weight of carbon-dioxide evolved per gram of material was the same for the unpollinated as for the pollinated specimens, every result which has been obtained shows a distinctly marked rise in the evolution of carbon-dioxide in the pollinated gynaeceums, as compared with the unpollinated.

The most strongly marked results were manifested in *Pelargonium zonale*, the amount of carbon-dioxide produced by the pollinated material being 5·8 times greater than the amount produced by the unpollinated. Pronounced differences were also observed, though to a less degree in *Cheiranthus Cheiri*, the pollinated specimens producing 2·0 times as much as the unpollinated, and in *Antirrhinum majus* the pollinated material evolved 2·6 times as much by weight of carbon-dioxide as the unpollinated.

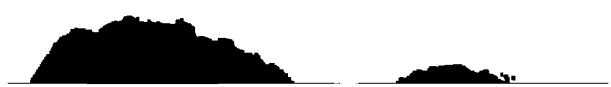
Throughout the series of experiments, with a few rare exceptions, the respiratory quotient in the pollinated gynaeceums exceeded that in the unpollinated, the principal exceptions occurring as isolated cases among the Monocotyledons. These irregular fluctuations, however, appeared to be independent of pollination, and, when they did occur, in no case did the respiratory quotient in the unpollinated specimens exceed that in the pollinated specimens to any great extent; the most notable instance being found in the *Canna*, where the respiratory quotient in the unpollinated specimens in one pair of experiments was in excess of that in the pollinated

specimens of the same pair by 0·05. The respiratory quotient in both kinds of material was somewhere below unity, except in *Pelargonium zonale*, in the pollinated gynaeceums of which it rose above unity, its maximum being 1·2, while in the unpollinated gynaeceums it fell well below unity.

Slight environmental changes, such as temperature and moisture in the atmosphere, or changes in the material itself, such, for instance, as would probably occur owing to the length of time which elapsed between the cutting of the flower and experimenting upon it, appeared to affect the oxygen absorption in a more marked degree than the amount of carbon-dioxide evolved by the material.

The net result of the whole work is to show that pollination not only produces a rapid rise of the respiratory activity, but also affects the respiratory quotient of the gynaeceum.

The foregoing work was carried out in the Botanical Laboratory at the Melbourne University. I wish to record my indebtedness to Professor Ewart for the interest he has taken, and for the advice he has given me.



The Value of Certain Nutritive Elements to the Plant Cell.[1]

BY

HOWARD SPRAGUE REED.

With two Figures in the Text.

I. INTRODUCTION.

IT is my purpose to describe in the following paper the results of a study upon the rôle of certain essential elements in the physiology of the plant cell. Since the times of De Saussure and Boussingault it has been known that the complete development of the higher plants and of many of the lower ones requires soluble salts of ten different elements, each one of which possesses a relatively low atomic weight. Some of these elements, like iron and magnesium, are required only in small quantities, but a certain amount is indispensable to practically all plants.

The studies of such investigators as Wolf, von Raumer, Loew, and others, have shown that certain of these essential elements accomplish more or less definite functions in the growth and fructification of the plant. There is every reason to believe that the different essential elements also perform more or less definite functions in the physiology of the cell. However, we have much less scientific evidence upon the latter subject than upon the former. Molisch ('95), Bokorny ('95), and Loew ('92, '99), have furnished some valuable data upon this question.

The majority of the workers who have studied the rôle of the essential elements in plant metabolism have worked upon the assumption that the almost exclusive function of these elements is to furnish chemical compounds suitable for elaboration into plastic and aplastic materials and fluids of the plant. As a result of my experiments I feel justified in advancing the opinion that the inorganic food constituents of the plant may also indirectly perform functions of the greatest value to the plant without necessarily entering into the composition of any of its parts.

The plant cell is an especially favourable object for studying the rôle of the essential elements, because it takes its food in the form of comparatively

[1] Contribution 15 from the botanical laboratory of the University of Missouri. A thesis presented to the Graduate Conference, 1907.

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simple compounds. The fact that the processes of metabolism in the plant cell are relatively slow, and permit of more exact observation than in the animal cell, makes the former a very favourable object of research.

De Saussure, in his classical 'Recherches chimiques sur la vegetation,' placed on record his observations and experiments, which proved that the ash of plants contains the same mineral nutrients which they drew from the soil. He showed that terrestrial plants draw their mineral nutrients from the soil in the form of aqueous solutions, and that the ash of seaweeds contains only the salts which are present in sea water. In certain respects De Saussure went far beyond his contemporaries. He succinctly states, for example, that plants do not absorb salts in the same proportion as they exist in the soil solution. He also recognized the ability of plants to utilize very dilute solutions of mineral nutrients.

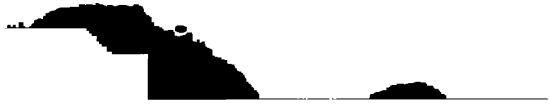
II. Historical.

1. Previous work upon the rôle of potassium.

De Saussure, in the treatise already mentioned, established the necessity of potassium salts for the growth of terrestrial plants. Birner and Lucanus ('66), experimenting with oat plants in water cultures, gave the first proof that the element potassium is absolutely indispensable for flowering plants, and cannot be replaced by rubidium, caesium, sodium, lithium, or ammonium. The perfection of the water-culture method and consequent refinement of experimental methods enabled these investigators to obtain data of fundamental value. Their work was extended and confirmed by G. Wolf ('68), who attempted to establish Liebig's law of the minimum for plants grown in water culture. The results of Hellriegel ('67) on barley also confirmed the observations of Birner and Lucanus. Molisch ('96) showed that the related elements, rubidium, caesium, lithium, and sodium, could not be substituted for potassium in the physiology of *Protococcus infusionum*. Nobbe ('70) seems to have been the first investigator to point out the necessity of potassium salts for the formation of carbohydrates in plants. Gaunersdorfer ('87) not only showed that lithium cannot be substituted for potassium but that it has a toxic action upon some plants.

Loew ('98 A) has shown that rubidium may replace potassium in the nutrition of certain of the lower plants. Also that rubidium exerts a stimulating action on plants when all necessary nutrients are present. Benecke ('07) has also stated that rubidium and caesium within certain limits may replace potassium in the nutrition of *Bacillus fluorescens* and of *B. pyocyaneus*.

In the presence of potassium, sodium has been shown also to produce increased growth in plants. Hellriegel ('98) found that plants grown in



sand cultures succeeded better when both potassium and sodium were present than when only potassium was furnished. Dassonville ('98), who studied the effects of various elements upon the form and structure of plants, found that wheat plants were strongly lignified at the base of the stem when sodium was substituted for potassium. He believed that potassium retards the lignification of the mechanical tissues, and that sodium, playing a less active rôle, is less favourable for growth and more favourable for lignification. Sodium was less favourable than potassium for the formation of secondary roots of the tomato. When sodium was furnished the number and importance of the vascular elements in the epicotyl were diminished, the cells of the cortex were larger and the intercellular spaces smaller.

Wheeler ('05) has shown that the addition of sodium salts to a soil already fertilized with a greater or less amount of potassium salts increased the growth of such plants as beets, radishes, and flat turnips, but did not increase the growth of rye, chicory, carrots, spinach, and lettuce. The continued application of sodium salts in the absence of any potassium salts was not generally beneficial.

The foregoing investigations indicate that there may be functions which either sodium or potassium may perform, and other functions which only potassium may perform. Such investigations are unfortunately open to one serious objection because conducted in soil or sand cultures. The soil, like any finely divided solid, possesses specific powers of absorption, and would hold a certain amount of basic bodies (among them potassium) in an absorbed state. The addition of sodium salts would tend to liberate some of the potassium with consequent benefit to the plant. The soils also furnish a certain amount of potassium from the potassium-containing minerals which enter into their composition.

The work of Breazeale ('06) is not, however, open to such criticism because conducted in water cultures. Breazeale was successful in measuring the amount of different nutrients removed from a complete nutrient solution after being transferred from solutions in which some one element was lacking. He showed that plants which had previously grown in solutions lacking potassium usually withdrew more potassium, in proportion to their growth, from the complete nutrient solution than plants of the same age which had previously grown in a complete nutrient solution. Plants which had previously grown in solutions lacking sodium also absorbed more potassium than those previously grown in complete nutrient solutions containing both sodium and potassium. Finally he showed that plants which had previously grown in solutions lacking both sodium and potassium drew most heavily upon potassium when placed into complete nutrient solutions. These results, more than any others known to the writer, appear to give definite facts upon which the question of the possible value

of sodium to the plant may be settled. They seem to point quite conclusively toward a possible utilization of sodium by the plant.

2. Previous work upon the rôle of phosphorus.

Ville ('61) appears to have been one of the first investigators to show conclusively that phosphorus is necessary for all plant-growth. Although his experiments were performed with sand cultures they show such definite results that there can be no doubt of his conclusions. He also showed that, in order to serve as a plant nutrient, the phosphorus must be in the form of phosphates.

Stoklasa ('96) and Molisch ('96) refuted the statements made by Bouilhac ('94) regarding the possible substitution of arsenic for phosphorus. The first-named investigator showed that such a substitution was impossible for phanerogams, the second confirmed the evidence for certain Algae.

Berthelot and André ('88) studied the amounts of phosphorus in *Amarantus caudatus* at different times during the growing season, finding that the relative content of phosphorus was greatest in the young plant, and decreased continually to maturity. Although it is universally held that phosphorus in the form of inorganic or organic compounds is necessary for the growth of any plant cell, little knowledge is at hand concerning the rôle of phosphorus in the cell. Loew ('91) described the pathological conditions ensuing when *Spirogyra* cells were deprived of phosphorus. The cells studied ceased to grow although they continued for some time to form starch and proteid ; the chloroplasts turned yellow, and fats and proteids accumulated in the cells. He also pointed out the importance of phosphorus for the formation of lecithin. He regarded lecithin as a body into which fat must be changed in order to become combustible in the protoplasm.

Becquerel ('04) found that moss spores would germinate and grow for about a month in nutrient solutions lacking phosphorus, but the protonema gradually lost its green colour and died.

Schoene ('06) reported the results of experiments upon the germination of moss spores in nutrient solutions lacking phosphorus. He observed that after sowing spores upon such deficient solutions that the oil content of the spore disappeared, and at the same time there were formed rod-shaped chlorophyll bodies. In *Funaria* the oil globules disappeared as the protonema developed. The more rapid the formation of starch, the more rapidly did the oil content vanish.

3. Previous work upon the rôle of calcium.

Aside from the earlier ideas upon the value and function of nitrogen in the plant, the first ideas upon the specific functions of any one of the mineral nutrients appear to have been those of W. Wolf ('64). That

investigator appears to have discovered, incidentally, that calcium salts have a stimulating action upon root growth. It has since been shown that other salts will produce the same effect, but Wolf's discovery appears as one of the first attempts to explain the unknown but beneficial action of calcium salts on certain soils.

Holzner ('67) assumed on very insufficient grounds that calcium salts are necessary for the formation of cellulose. He regarded calcium as the carrier of phosphoric and sulphuric acids in the plant, and believed that these acid radicals were replaced by that of oxalic acid when they reached cells in which proteid synthesis occurred. We have evidence now that this latter assumption must be regarded as untenable. G. Wolf ('68) first showed that the functions of calcium in the plant could not be performed by magnesium. Stohmann ('62) demonstrated the necessity of calcium in the formation of the green parts of the plant by cultivating corn plants in solutions lacking calcium. At the end of five weeks the plants were dead at the tips; after the addition of a small amount of calcium salts, the plants quickly took on new life and began forming new leaves and shoots.

Boehm ('75) seems to have been the first to discover any of the more important functions of calcium. He observed an abnormal accumulation of starch in plants (*Phaseolus vulgaris*) grown in water cultures lacking calcium; the accumulation of starch being in the pith and cortex of the lower part of the stem of the plants. He also found that calcium is necessary for the formation of new cell walls, although his idea of the mode in which calcium functions in cell-wall formation was probably incorrect. He believed that the function of calcium in the formation of cellulose walls was similar to its action in the formation of bone in animals. It was demonstrated by Molisch ('95) that this conception of the action of calcium was erroneous. Molisch showed that transverse walls in *Spirogyra* did not develop, or consisted only of imperfect septa when calcium salts were lacking, and that there was a non-formation of all parts. Boehm also found that the addition of calcium salts to distilled water prolonged the life of the plants which grew in it; but that when magnesium instead of calcium salts were added they exerted a toxic influence upon the plants.

The next work of importance upon the function of calcium appears to have been that of von Raumer and Kellermann ('80), which especially advanced the knowledge of the rôle of calcium in the transport of carbohydrates in the plant. Von Raumer ('83) carried their investigations still further and published them in a paper of fundamental importance. He seems first to have discovered the necessary ratio between calcium and magnesium. When plants were grown in distilled water, or in solutions lacking both calcium and magnesium, they made better growth than in solutions where only calcium was lacking. He showed that magnesium had a toxic action which could be counteracted by calcium. The plants (*Phaseolus*

multiflorus) which he employed displayed, when grown in the absence of calcium, interruptions in the translocation of carbohydrates. When magnesium was lacking the chlorophyll bodies became unhealthy, and, as a result, the plants possessed an etiolated appearance.

Loew ('92) ascribed a highly important function to calcium. He regards calcium as one of the important mineral bases which enter into the constitution of the proteids composing the cell nucleus and chlorophyll bodies. According to his interpretation these proteids take up magnesium in the absence of calcium, and the resulting magnesium-proteid compound has not the same well-defined capacity for imbibition and other processes which the calcium-proteid bodies possess. Hence the harmful effects observed when plants grow in a calcium-free medium which contains magnesium. Loew verified the observations of Boehm and von Raumer that solutions of pure magnesium salts have a toxic action, and ascribed it also to the harmful substitution of magnesium for calcium in the proteids. Bokorny ('95) repeated and confirmed many of Loew's experimental data. Unfortunately this theory fails in the case of a group of plants which form considerable amounts of proteid, viz., the Fungi. It is well known that the members of this group of plants are able to reach maturity and form reproductive bodies in the entire absence of calcium. More conclusive evidence would seem to be needed to establish the calcium-proteid theory.

In their study of *Phaseolus* plants grown in water cultures, von Portheim and Samec ('05) obtained results which afford some support for the calcium-proteid theory. They found by analysis that normal plants contained much more calcium than magnesium ; but plants grown in solutions lacking calcium had a much greater proportion of magnesium than the normal plants. In pathological conditions induced by the absence of light, the amount of magnesium in the plant was nearly equal to the calcium, although the plants were supplied with a complete nutrient solution. The fact that the plants deprived of calcium took up more magnesium strengthens Loew's assumption of the calcium-proteid compound, yet it will require further investigation to determine whether this additional magnesium may not find use in other ways in the plant. Haselhoff ('93) showed that strontium was unable to replace calcium in water cultures of *Vicia* and *Zea*. Loew ('98) and Suzuki ('00), have also shown that the functions of calcium cannot be performed by strontium or barium.

The neutralizing action of calcium in the synthesis of proteids has been taken up anew by Yermakov ('05) in connexion with the assimilation of nitrates. This author assumed that since ammonia and oxalic acid are formed when nitric acid acts upon glucose, that a similar reaction might occur in the synthesis of proteid in the plant. If this assumption were true, the oxalic acid formed would not be neutralized in the absence of calcium salts, and being injurious to the plants would hinder the assimi-

lation of nitrate nitrogen. Experimental data verified such an assumption. Leaves which were supplied with calcium salts were able to assimilate nitrates, but where no calcium salts were furnished the nitrate remained unaffected.

Schimper ('88) concluded from a study of the green parts of the plant that the principal function of calcium was to precipitate oxalic acid and soluble oxalates. As the same author found (Schimper, '90) in a subsequent research that calcium is not always present in the meristem, he assumes that potassium may sometimes take the place of calcium in neutralizing oxalates.

Groom ('96), from a study of interrupted carbohydrate translocation in the absence of calcium, concluded that the presence of acid potassium oxalate is inhibitory to the action of diastase. This hypothesis is strengthened by the observation of Hammersten ('96) on the need of calcium salts for the formation of blood clots. Calcium salts appear to play a specific part in forming blood clots which is hindered by the alkali oxalates. It is quite possible that this action stands in the closest relation to the formation of the fibrin ferment.

Additional data have recently been presented by Grafe and von Portheim ('06), which confirm the idea that part of the injury caused by the absence of calcium is due to the interference in carbohydrate transformation and transfer. These investigators found that the addition of levulose, dextrose, or saccharose to calcium-free solutions enabled *Phaseolus* plants to live in them for a longer period than in calcium-free solutions containing no sugars.

Kraus ('97) brought out evidence on the other hand that the importance of calcium does not depend primarily upon its power to precipitate soluble oxalates since some plants are able to tolerate the presence of oxalic acid in their tissues. He found that the supply of calcium oxalate in the rhizomes of certain plants was drawn upon when other sources of calcium were gone. Loew ('98) also doubted the necessity of calcium to neutralize oxalic acid; but Bruch ('02) has gone farther and shown that the Gramineae and some other plants can endure relatively large amounts of oxalates. Between concentrations of 278 and 18 parts per million of oxalates wheat plants grew to the period of blossoming (seven weeks). In that length of time all the oxalates furnished them had vanished, although it is possible that micro-organisms in the cultures may have aided in their destruction. He found that wheat could be grown in distilled water containing calcium oxalate and in nutrient solutions where calcium sulphate was replaced by calcium oxalate.

In view of these facts the necessity of calcium to neutralize oxalic acid does not seem to be as imperative as was held by Schimper, Groom, and others.

4. Previous work on the rôle of magnesium.

Boehm ('75) seems to have been almost the first one to point out any specific action of magnesium salts upon plants. It was quite natural that he should have discovered the toxic action of magnesium salts in solutions when he attempted to use these salts in water cultures. Von Raumer ('83) went further and pointed out the antidoting power of calcium for magnesium, and the ratio of the two elements necessary for the best growth of plants. He observed that *Phaseolus* plants suffered characteristic injury when grown in the absence of magnesium. After becoming about one meter high the internodes of the plants ceased to elongate, and the new leaves remained small and chlorotic.

The apparent toxic qualities of magnesium salts have already been mentioned in discussing the action of calcium salts, but other considerations necessarily come up at this place. Loew holds the view, previously mentioned, that magnesium salts cause injury as a result of displacing the calcium ions from certain important proteid compounds, and that the resulting magnesium compound is not capable of performing the normal functions necessary to the continued existence of the living tissues. According to his view a proper ratio of calcium and magnesium salts should be afforded, if the normal processes of the cell are to continue.

In this regard the recent paper by Duggar ('06) is of value. In a careful study of the toxic action of various salts *added* to sea water, he found that magnesium salts had a very low toxicity for marine algae. Granting, as we must, in the light of our present knowledge, that the marine algae are able to withstand much greater concentrations of magnesium salts than fresh water algae, or phanerogams, it is difficult to see how this great group of plants (some of them possessing a truly high organization) conforms to the general statements made by Loew, concerning the replacement of calcium by magnesium in the hypothetical calcium-proteid compound. Duggar found that ammonium salts had a greater toxic effect than any other basic radical which is ordinarily regarded as a nutrient for an autotrophic plant. Arranged in the order of their toxicity for marine algae, his paper gives the series NH_4, K, Ca, Mg. He states further, 'The very low toxic effect of the neutral magnesium salts upon the marine algae makes it evident that these plants are very notable exceptions to the rule which Loew and others have found to hold in the nutrient relations of a few fresh water algae and many phanerogams.' The great preponderance of magnesium over calcium in the experiments made by Duggar shows that the protoplasm of these plants is not seriously injured by magnesium.[1]

[1] Loew has suggested that such plants are able to withstand large amounts of magnesium on account of the acidity of the cell sap. It is difficult to see how the acidity of the cell sap can

It would seem that this power of calcium is that of a specific antidote for magnesium, just as specific antidoting properties have been demonstrated between various other elements.

The antidoting power of calcium was demonstrated by Kearney and Cameron ('02). In an extensive series of experiments they found that calcium nearly always had marked ability to decrease the toxicity of both magnesium and sodium salts for seedlings of *Lupinus albus*. Krönig and Paul ('97) have shown that a similar marked decrease of toxicity for bacteria was obtained when the chloride of sodium, potassium, magnesium, zinc, or cadmium was added to a solution of mercuric bichloride. Clark ('01) demonstrated that the toxic value of mercuric bichloride for fungi was greatly diminished by the addition of sodium chloride or hydrochloric acid. Pigorini ('07) has even been able to show that such a poisonous substance as sodium thiosulphate was able to diminish the toxicity of silver nitrate solution for *Carassius auratus*. True and Gies ('03) have shown that the toxicity of salts of the heavy metals to seedlings is greatly reduced by the addition of salts of sodium, magnesium or calcium. Loeb ('06) has found that the effect of magnesium in producing rhythmical motions in a jelly-fish may be inhibited by the addition of an equivalent amount of calcium or potassium to the water. Osterhout ('06) has reported that pure sodium chloride solutions are quite toxic to certain marine plants. The toxicity is much diminished if calcium chloride be added, and is still further diminished if both calcium and potassium chlorides be added to the sodium chloride solution.

While it is no doubt true that a very large number of plants require that some sort of a ratio between calcium and magnesium be maintained, it is difficult to fit the theory of the necessary calcium-proteid compound to a vast number of plants, which normally live with little or no calcium. It seems more plausible to postulate (and at present it appears impossible to do more) that calcium salts are able to perform some specific antidoting powers against magnesium in the plant. There appear to be instances where the antidoting may be performed by sodium, or by potassium as well as by calcium. Future investigation will have to determine the nature of this antidoting power.

Mayer ('69), Raulin ('69), and Winogradsky ('84) demonstrated that yeast and certain other fungi could grow in the absence of calcium, but required magnesium. Molisch ('95) showed that the same requirements held for *Microthamnion*, *Stichococcus* and some other Algae. Sestini ('91) showed that the functions of magnesium could only be imperfectly performed

make any particular difference to these or any other plants unless, indeed, it be proved that phosphoric acid is relatively abundant. On the other hand it is possible that the comparatively large amount of sodium present in the sea water might act as a partial antidote for magnesium.

by beryllium, although this element may be absorbed in rather large quantities.

Loew ('92), in discussing the rôle of magnesium in the plant, set forth the hypothesis that one of its functions was to serve as a carrier for phosphoric acid. On account of the readiness with which magnesium salts are dissociated, they would present the phosphoric acid radical in a form favourable for assimilation. The results of numerous analyses can be cited to show that magnesium is usually abundant in the plant where nucleoproteid is being formed. If magnesium has such a function, it is evident that a small quantity of magnesium could be used for a great deal of work, since it may serve repeatedly as a carrier of phosphoric acid to the cell. This would also explain such an observation as that of von Raumer, that *Phaseolus* plants were able to grow to a height of one meter when they were supplied only with the magnesium stored in the seed.

Bokorny ('95) practically duplicated Loew's results upon different algae. In addition, he stated that the nuclei of cells which had lived in solutions lacking magnesium were smaller than those of the cells in control cultures. His observations were principally upon the modifications produced in the nuclei and chloroplastids.

III. The Technique of Experimentation.

The experimental study described in the following pages was entered upon in the year 1904, in the botanical laboratory of the University of Missouri. To Professor B. M. Duggar, under whose general oversight this investigation has been prosecuted, I wish to extend my heartfelt thanks for his uniform kindness in freely giving advice, suggestion, and kindly criticism of immeasureable value. Any adequate expression of my indebtedness to him is impossible. I wish also to express my sincere gratitude to Professor Waldemar Koch for valuable suggestions, and for material which he so generously placed at my disposal.

1. Choice of suitable plants for cultures.

In choosing material suitable for a study of the action of different elements in cell metabolism, I found it desirable to take those plants which would admit of microscopical examination in the living condition. For certain purposes it was also necessary to employ plants which contained a minimum of reserve food substances. With these and other considerations in view I have employed different species of filamentous Algae, the protonema of moss, the young prothalli of ferns, the root-tips of phanerogamic plants, and the filaments of *Basidiobolus ranarum*. The filamentous thalli of the algae could be advantageously studied in the living condition under the microscope, and the species of *Spirogyra* employed were readily fixed

stained and converted into permanent preparations for more detailed study. The same was true of the prothalli of the ferns employed. The root tips of young chlorophyll-bearing plants which were always grown in water cultures were studied only after being fixed, sectioned and stained. The filaments of *Basidiobolus* yielded little when studied in the living condition, but when fixed and stained they permitted of accurate study with the higher powers of the microscope.

With each plant experiments were conducted which were designed to show the rôle of certain elements in the physiology of the cell. This was accomplished, in most cases, by using a series of solutions which lacked, in turn, one of the essential elements; the missing radical being replaced by a non-essential and non-toxic (at least at the concentration employed) radical. After living in the deficient solution for a varying length of time, depending both upon the nature of the plant employed and the purpose of the experiment, the plants were studied microscopically and compared with the cells of a like plant which had lived for a similar time and under exactly similar conditions in a complete nutrient solution.

2. Preparation of nutrient solutions.

In preparing the nutrient solutions, little difficulty was experienced in obtaining chemically pure salts of the standard chemicals.

A supply of pure non-toxic distilled water was not, however, always easy to obtain. Ordinary distilled water is seldom suitable for physiological work, because it contains traces of the metals which compose the distilling apparatus. Usually copper is employed in some form in the construction of the apparatus, and the traces of this metal in the distilled water have been recognized as sources of error for some time. The use of block tin apparatus reduces the amount of metallic material in distilled water to practically *nil.* Apparatus of glass was used in most of the redistillation which I performed, but since the alkali silicates are quite soluble, the water obtained from such apparatus contained more or less alkali salts.

Alkaline permanganate was usually added to the distilling flask; and a satisfactory grade of distilled water was usually obtained. While the experimental work was still in progress, a method was devised by Livingston ('07) which gives a non-toxic distilled water. By the use of a finely divided solid, substances are removed by absorption which are extremely difficult to remove by distillation. To obtain such physiologically pure water, I used freshly prepared ferric hydroxide, as an absorbing agent, which was made by adding ammonium hydroxide to a hot solution of ferric chloride so long as a precipitate was formed. The excess of ammonium hydroxide was boiled off, and the ferric hydroxide was filtered out. The precipitate was thoroughly washed on the filter with hot water. About 350 cc. of the moist ferric hydroxide was added to a bottle con-

taining 1,500 cc. of redistilled water, and shaken for several minutes. The bottle was allowed to stand until most of the ferric hydroxide had settled to the bottom, then the supernatant water was passed through a filter paper and used for making up solutions.[1]

When stock solutions were necessarily kept for some length of time, they were stored in flasks of Jena glass closed with rubber stoppers. Where I desired to cultivate plants in the absence of potassium, the glass-ware to be used for the cultures was filled with a solution of potassium hydroxide. After standing for several weeks, the glass-ware was thoroughly washed out with water, and steamed for several hours in an Arnold sterilizer. By means of this treatment it is believed that the error of experiment due to soluble bases in the glass was reduced, but not entirely eliminated. It has been shown by Kohn ('05) and Benecke ('07) that the growth and pigment formation of certain bacteria is influenced by small amounts of the essential elements contained in the glass. It need not be assumed, however, that the growth of algae or of phanerogams would be as greatly influenced by the presence of these salts, as bacteria have been shown to be. I tried to use vessels lined with a coating of paraffin as described by Molisch ('95), but without success. The paraffin contained petroleum products which had a toxic effect upon the plants.

The solutions for experiments were prepared by mixing proper quantities of the stock solutions, and diluting to the proper concentration.

[1] My experiments with water prepared in this manner confirm the experience of Livingston ('07) that such distilled water gives a superior growth of plants when used as the basis for a culture solution. These results are in harmony with the work of Lyon ('04) in which he found that tap-water which was decidedly toxic to *Arbacia* eggs was much improved by boiling away one-third of its volume. When the water was distilled from glass into glass the first, second, and third tenths of the distillate exhibited toxic properties, the fourth portion was of good quality. The best quality of distilled water was obtained by distilling in glass with potassium bichromate and sulphuric acid added to the contents of the retort. A theory has recently been propounded by Schreiner and the writer ('07) to explain the toxic properties of some distilled waters. It has been shown that even when distilled in glass apparatus many soil extracts give water unsuitable for the growth of plants. This is probably due to the existence of toxic substances in the soil extract which are volatile and pass over with the water vapour. These toxic substances may often be boiled off with resulting improvement to the soil extract. All waters from wells, springs, rivers, or lakes are virtually soil extracts. Upon distillation the volatile toxic substances may pass over with the steam, are condensed, and appear in the distillate. It is true that repeated distillation in the presence of active oxidizing or reducing agents will remove such substances, but they are more easily and innocuously removed by treatment with an absorbing agent like ferric hydroxide. Rain-water gathered in glass vessels is not toxic to plants. This good quality is to be accounted for by the fact that such water remains for some time in the vapour condition, and as such is subjected to the action of air, light, and other agents capable of effecting oxidation. The action of these agents is probably the same as that of the oxidizing agents added to the retort of the distilling apparatus.

3. Advantages of certain formulae for nutrient solutions.

In the first experiments upon algae and fern prothalli, I used the well-known formula of Knop in making the nutrient solution:—

Calcium nitrate	4 g.
Potassium nitrate	1 g.
Magnesium sulphate	1 g.
Potassium dihydrogen phosphate	1 g.
Ferric chloride	trace.
Distilled water	7 litres.

Repeated trials of this solution agreed in showing that it is unsuited for the growth of algae. A large quantity of nitrate is supplied in the form of calcium nitrate. The algae use the nitrate more rapidly than the calcium. As a result, the solution continually increases in alkalinity, and thereby becomes less and less suitable for the growth of Algae. A neutral or slightly acid medium is more favourable for the development of most algae, and this condition was fulfilled in the original solution by the presence of the potassium dihydrogen phosphate. Unfortunately this condition is not maintained after the plants begin to grow, because the nitrate is removed from calcium nitrate, resulting in alkalinity due to calcium carbonate formed with the carbon dioxide always present in such cultures. The lower algae will thrive, as Molisch ('96) stated, in a slightly acid medium, and it is probable that the higher algae grow best when the solution is near the neutral point. Klebs ('96) found that a distinctly alkaline culture solution was necessary for the formation of zoospores in *Vaucheria*. The conditions favouring vegetative growth were not determined with respect to acidity.

After discarding Knop's formula, I employed the following modification of a formula of Beijerinck given by Moore ('03):—

Ammonium nitrate	0·5 g.
Potassium dihydrogen phosphate	0·2 g.
Magnesium sulphate	0·2 g.
Calcium chloride	0·1 g.
Ferric chloride	trace.
Distilled water	1,000 cc.

The nutrient solution when made according to this formula, has a concentration of 1,000 parts per million (0·1 per cent.). This concentration is greater than necessary, in fact is much greater than that to which most plants are accustomed in nature. Though a more dilute solution would undoubtedly serve just as well, I have not made any attempts to determine the limits of concentration for optimum growth.

This formula proved to be eminently satisfactory for the growth of

algae, moss protonema, and fern prothalli. Ammonium nitrate appears to be a very suitable form in which to supply a source of nitrogen. The plant probably derives benefit sooner or later from the reduced form of nitrogen as ammonium, as well as from the oxidized form as nitrate. There are undoubtedly different processes of metabolism for which each is better suited. This solution does not tend to become more unsuited for algae, the longer they grow in it. The presence of potassium dihydrogen phosphate gives the solution a faintly acid reaction. If the potassium phosphate has passed entirely into solution before the calcium chloride is added, there is practically no precipitation of calcium phosphate until heat is applied in the process of sterilization. This precipitate did not prove troublesome, however, because the solutions were measured out before being sterilized, and errors in measurement due to the presence of a solid substance were avoided. The calcium is furnished as calcium chloride in a much smaller quantity than in the solution of Knop. This smaller amount must be regarded as more rational if we regard the needs of the plant. As the calcium radical is used more rapidly than that of chlorine, the solution tends to become slightly more acid, and the conditions are correspondingly better for algae.

The nutrient solution which was used in the cultivation of phanerogams was one given by Pfeffer (Physiology of Plants, English trans., vol. i, p. 420):—

Calcium nitrate	4 g.
Potassium nitrate	1 g.
Magnesium sulphate	1 g.
Potassium dihydrogen phosphate	1 g.
Potassium chloride	0·5 g.
Ferric chloride	trace.
Distilled water	3 or 7 litres.

This solution gave good results with phanerogams, which are apparently favoured when the reaction of the solution is slightly alkaline. It is somewhat more concentrated than necessary, especially when only three liters of water are used. At that dilution the solution would have a concentration of 2,500 parts per million (0·25 per cent.); when seven liters of water are used the solution has a concentration of 1,070 parts per million (0·107 per cent.). This solution is somewhat more concentrated than necessary for the growth of higher plants. Indeed, the work of Breazeale ('05) indicates that the optimum concentration of salts for wheat plants in water culture is approximately 300 parts per million.

The volume of the solution employed in cultures was always made large. The advantages of a large volume are numerous; the concentration of the solution is altered more slowly as the plants remove the nutrients;

again, the deleterious products which arise as excretions are more highly diluted, and hence less harmful to the plants.

When placed in the culture flasks the solutions were sterilized with steam in an Arnold sterilizer. A single heating lasting about forty minutes was usually sufficient for the sterilization of the aqueous solutions.

The media upon which the *Basidiobolus* was grown require special mention. I found that for my purposes an aqueous medium was unsatisfactory, and was obliged to add agar agar to all nutrient solutions employed. The formula first used is one which is commonly used in physiological work with the Fungi:—

Ammonium nitrate	1 g.
Potassium dihydrogen phosphate	0·5 g.
Magnesium sulphate	0·25 g.
Cane sugar	5 g.
Distilled water	1,000 cc.
Agar agar	2·5 g.

The growth of the fungus on this culture medium was not entirely satisfactory, since the palmella stage described by Raciborski ('96) is produced when sugar is supplied as a source of carbon. The filamentous stage was produced when starch instead of sugar was supplied as the source of carbon. I prepared a paste by cooking in an Arnold sterilizer 115 grams of clean chopped potato in 400 cc. of distilled water. The inorganic salts and agar were dissolved in this paste in the same proportions given above. This gave a medium upon which *Basidiobolus* produced good filaments.

4. Microchemical Methods.

In examining living cells under the microscope, it was often necessary to apply some reagent in order to determine the nature of the substances found in the cells. The reagents employed were usually immediately fatal to the cells, but they were the best means of showing the nature of substances produced under the different conditions of metabolism. It is believed that the employment of such reagents gives a more accurate representation of the actual products of cellular activity, than the employment of the ordinary fixing and staining reagents.

Gram's solution of iodine in potassium iodide was used for identifying starch and erythro-dextrin. With the latter it gives a diffuse red-pink colour which can be observed microscopically.

The identification of albuminous substances was rather uncertain when the biuret test was used, because the colour was never deep enough to be very intense, and hence difficult to observe with the microscope. For microchemical work, I found the method described by Loew and Bokorny ('89) to be more satisfactory. The method as I employed it was as follows:

the living cells to be examined were placed in 0·1 per cent. ammonium hydroxide for one hour, then into a 10 per cent. solution of potassium ferrocyanide containing 5 per cent. acetic acid. The mixture was always freshly prepared by mixing equal quantities of 20 per cent. potassium ferrocyanide, and 10 per cent. acetic acid. The cells remained in this mixture for twelve hours, and were then washed in distilled water until the washings no longer gave a blue colour with ferric chloride. They were then placed in a 3 per cent. solution of ferric chloride for twelve hours. After this treatment the albuminous materials were coloured deep blue.

In testing for fats I did not rely upon the usual application of one per cent. osmic acid. The black colour which appears when dilute osmic acid is applied to fats follows from the reduction of osmic acid (osmium tetroxide) to metallic osmium. This reduction is accomplished by the unsaturated series of fats represented by olein and oleic acid. Wlassak ('98) has shown that the same reduction of osmic acid may be accomplished by lecithin. This may happen by virtue of the fact that lecithin contains the oleic acid group, lecithin being in fact the stearic-oleic-glycero-phosphate of cholin, and in the presence of water some oleic acid is probably formed. This is in accord with Wlassak's observation that pure lecithin readily stains with osmic acid if previously kept for a time in water. Wlassak also pointed out that while osmic acid stains both fat and lecithin, the method of Marchi stains only the fat. This is because the lecithin loses its power of reducing osmic acid if it is kept for some time in a solution of potassium bichromate, while olein does not. Halliburton and Mott ('02) have given this subject further study, and point out the probability that when potassium bichromate acts upon lecithin it oxidizes the oleic group. When osmic acid is applied to such a preparation it is not reduced, and hence the preparation does not blacken. In practice I found it best to put the plant tissues directly into Erlicki's fluid, and allow them to 'harden' five to seven days at room temperature. Subsequently they were treated with one per cent. osmic acid.

Although no extensive experimentation was attempted with stains and mordants, a few were of sufficient merit to deserve mention.

Excellent results in staining Algae were obtained with iron-alum-haematoxylin and with safranin. The safranin stain was much improved when the filaments were previously mordanted in potassium permanganate according to the method of Henneguy.

In staining the protonema of mosses and the prothalli of ferns, the best results were obtained by using a modification of Hartog's nigrosin and carmin method.[1]

[1] The process is as follows:—(1) The living material is put for forty minutes in 50 per cent alcohol containing glacial acetic acid equivalent to two per cent. of the entire volume. (2) After briefly rinsing in 50 per cent. alcohol, the material is stained thirty to forty minutes in Grenacher's

For staining the microscopical sections of root tips, the best results were obtained from the use of Mann's Eosin and Toluidin Blue, according to a method which I have described elsewhere (Reed, '04).

IV. Data obtained from Experimental Work.

1. Experiments upon the rôle of potassium.

The paramount importance of potassium for the production of carbohydrates in the plant, as well as for the synthesis of proteids, renders its study of primary interest. My results agree with those of other investigators in showing that potassium is indispensable for the continued growth and normal functioning of plants. Even when carbohydrates, like sugar, are supplied to most Fungi, they are unable to form proteids in the absence of potassium, except in the few instances where rubidium may be substituted for it.

From the standpoint of the experimenter, it is difficult to ascertain what will happen when plants are totally deprived of potassium salts. In the first place there is always a small amount of potassium in some form in the seed or spore from which the plant originates. Knowing, as we do, the power of the plant to use repeatedly the same supply of potassium for different purposes, one must not disregard the value of a small supply of that element. In the section of this paper dealing with methods, it has been already indicated that the small amount of potassium in the glass-ware which is continuously soluble is capable of partially supplying the needs of the plant grown in a culture solution in a glass vessel.

In a series of cultures of moss protonema where potassium was purposely omitted from part of the solutions, I eliminated, so far as possible, the amount of potassium originally present. Specimens of a moss (Atrichum sp.) bearing mature sporogonia were collected in January. They were kept in a dry condition in the laboratory until March 9, when they were used in making cultures. By means of sterile pincers and needle, the contents of mature capsules were scraped out upon a sterile slide, and at once transferred to the culture solutions. The cultures stood before an east window in light of moderate intensity, where the temperature ranged between 8° and 15° C.

At the expiration of a month the spores in the control cultures were found to have germinated, and to have produced healthy green protonemata varying from 2 to 5 millimeters in length. The filaments of the protone-

borax carmin. (3) It is then stained for two and one-half to three hours in a strong blue-black solution of nigrosin in 50 per cent. alcohol which contains acetic acid equivalent to 5 per cent. of the entire volume. (4) Decolorization is effected with 70 per cent. alcohol; (5) then dehydrate with absolute alcohol, clear in clove oil and mount in balsam. If the amount of material is small the first two processes may be carried through on a slide, but the third is best done in a watch-glass or other covered receptacle.

mata were profusely branched, and showed evidence of rapid growth. The cells were filled with bright green chlorophyll bodies which contained starch. The spores in the solution in which sodium had been substituted for potassium had germinated, but none of the protonemata had grown to a length exceeding twice the diameter of the original spore. Starch could not be demonstrated. In solutions where no other metal of the alkali group had been substituted for potassium,[1] none of the spores had germinated, nor even shown the characteristic green colour which precedes germination.

Two months after setting up the cultures, the results were found to bear practically the same relations to each other. The protonemata in the complete nutrient solutions had made excellent growth. The cultures in which sodium had been substituted for potassium showed a great many spores which had germinated, and produced moderately well developed protonemata. The protonemata in this solution had a very characteristic appearance, owing to the fact that most of the individual cells were short and swollen, giving the filaments a moniliform appearance (see figure 1). The lack of potassium did not appear to be detrimental to the development of chlorophyll, but I was unable to demonstrate the presence of starch in any of the chloroplastids.

The outcome of these experiments seems to indicate quite clearly that although potassium is absolutely essential for the proper functioning of *all* the processes of metabolism in the cell, there are certain processes which may go on when potassium is present in sub-minimal amounts, *provided that sodium is also present.* The germination and partial development of the moss sporelings illustrates this principle. When no sodium was supplied it was found that there was still less growth than where sodium was substituted for potassium. Further illustrations of this principle are shown by the work of Breazeale ('06) upon the higher plants. Another indication that the sodium is of some value is shown by the work of Becquerel ('04), who found that spores of *Hypnum velutinum* would germinate and grow for a time upon nutrient solutions containing no potassium salts. That there are some biological factors which play a part in this problem, is

[1] The solution in which sodium was substituted for potassium had the following composition :—

Sodium nitrate	0·5 g.
Sodium dihydrogen phosphate	0·2 g.
Magnesium sulphate	0·2 g.
Calcium chloride	0·1 g.
Ferric chloride	trace
Distilled water	1,000 cc.

The solution in which neither sodium nor potassium were furnished had the composition :—

Ammonium nitrate	0·5 g.
Diammonium hydrogen phosphate	0·2 g.
Magnesium sulphate	0·2 g.
Calcium chloride	0·1 g.
Ferric chloride	trace
Distilled water	1,000 cc.

indicated by the fact that Becquerel was able to germinate spores of *Hypnum velutinum* on potassium-free solutions, but not spores of *Atrichum undulatum.*

The results of my experiments also show that potassium is intimately connected with the formation of carbohydrates, and that the lack of starch is not simply due to a pathological condition, because the chloroplastids remained normal for two months, and even increased in numbers. Evidently the sub-minimal amount of potassium was sufficient for the manufacture of the proteids necessary for the formation of plasmatic material. This phase of the cell's activity will be treated more fully in succeeding paragraphs.

The relation between potassium and starch formation was also studied by cultivating prothalli of *Gymnogramme sulphurea* upon the surface of

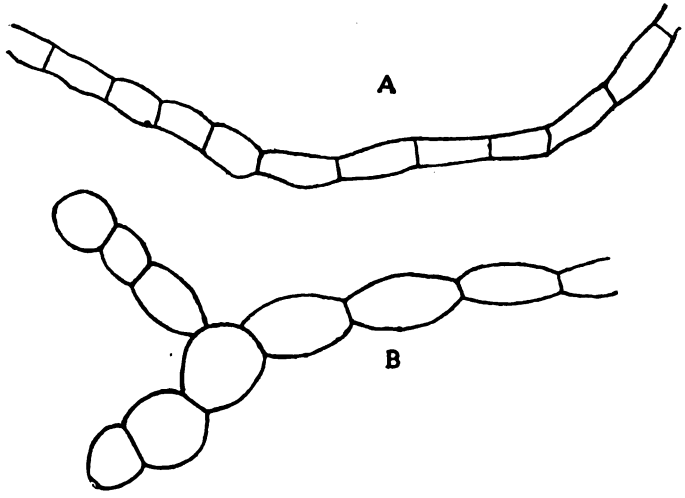


FIG. 1. Effect of absence of potassium on cells of Moss protonema. A. Filament from a complete culture-solution. B. Filament from a culture-solution lacking potassium. Drawn with aid of a camera lucida. Same scale of magnification used in both cases.

nutrient solutions. On April 15 I made cultures in solutions lacking potassium. In part of them sodium had been substituted in an amount equal to the potassium taken out, in others neither sodium nor potassium was present. Spores were also placed in dishes of full nutrient solution, and in dishes of distilled water. At the expiration of eighteen days, all the spores except those in distilled water had germinated and grown out into short filaments. In the distilled water cultures there were no evidences of growth beyond the formation of a few rhizoids. At the end of thirty-three days the prothalli in all solutions lacking potassium were much alike. At the end of three months there was noted a slightly better growth in the

cultures where sodium had been substituted for potassium than where neither one was present. The plants appeared to have obtained sufficient potassium from the glass vessels to enable them to grow and form chloroplastids, but they were devoid of starch.

Selecting a dish containing prothalli growing in a solution containing neither potassium nor sodium, and hence devoid of starch, I added a few drops of a 1 per cent. solution of potassium phosphate. When examined eleven days later, they were found to contain a large amount of starch. This experiment also goes to demonstrate the necessity of potassium for the formation of starch in the cell, although that cell may contain healthy, growing chloroplastids.

While it would be unsafe to apply *in toto* the conclusions obtained from the study of marine Algae to that of fresh-water Algae, it is interesting to note the results of Arber ('01 *a*). This investigator reported that sodium salts (preferably the chloride) are necessary in order for the formation of starch in *Ulva latissima*. The presence of potassium chloride seemed to inhibit almost completely the formation of starch, especially if present in an amount greater than that of normal sea water.

When *Hydrodictyon* was cultivated in solutions lacking potassium, the harmful effects were more evident on the protoplasm than on the starch-forming powers of the cell. When neither potassium nor sodium was present the cells lived longer than where sodium was substituted for potassium. In the injured cells the protoplasm often had the form of a reticulum on the inner surface of the cell-wall. Usually the reticulations began to form at the end of a cell, and often formed simultaneously in two or three cells which abutted upon each other. The application of iodine solution showed the presence of erythrodextrin in cells which had died, and considerable amounts of starch in cells which were still alive.

Cells of *Basidiobolus ranarum* cultivated on potassium-free media exhibited protoplasmic structures which were distinctly more vacuolate than those of the control cultures. The nucleoli were less distinct than those of the control cultures.

In another experiment *Zygnema* filaments were cultivated in solutions lacking potassium. The pyrenoids of the chloroplasts rapidly lost their starch, and at the end of eight weeks the chloroplasts themselves showed signs of serious injury. The chloroplasts had become intensely vacuolated and the radiating fibrillae of protoplasm which normally suspend them had disappeared.

Instructive results were obtained from the study of cells which had been stimulated to divide karyokinetically in the absence of potassium. It is well known that cells in the filaments of *Spirogyra* may be stimulated to divide by exposing them to a low temperature in the dark, and then transferring them to optimum conditions of heat and light. The cultures

I used were placed in a refrigerator at a temperature of 5° to 10° C. during the night. The next morning they were removed and placed before a window in strong diffuse light at a temperature of 22° to 25° C. After two or three hours there was usually little difficulty in finding numerous cases of mitosis in the control cultures. Five hours after the material had been taken from the refrigerator, most of the mitotic divisions were completed, and the new cell-wall entirely formed.

In carrying out these experiments I chose cultures of *Spirogyra* which had grown for thirty-five days in the absence of potassium salts, together with a number of control cultures which had grown for an equal length of time in complete nutrient solution. Both series of cultures were subjected to conditions which induced mitotic divisions in the control plants. The filaments in the potassium-free cultures appeared to be in a living condition, although by the absence of starch it was plainly to be seen that they felt the lack of potassium salts. The comparatively healthy condition of the filaments may be accounted for by the fact that during much of the time that they had been deprived of potassium the temperature had been between 12° and 18° C. At that temperature there would be less activity in starch formation, consequently the absence of potassium was less severely felt.

When the cells were exposed to conditions normally inducing mitotic division, the lack of potassium was very evident. A short time after they had been placed in optimum conditions it was found that the cells in the potassium-free solution had elongated to at least twice their normal length. The nuclei also had elongated in the direction of the longitudinal axis of the cell. A careful examination of both living and fixed material from these cultures failed to show any instances of cell or nuclear division. At the same time there were numerous instances of mitotic division in the control plants. In the species of *Spirogyra* which was employed the average length of the cells was between 0·4 and 0·5 mm. The average length of twenty cells which had been stimulated without being able to divide was 0·85 mm., while one cell was found which measured 1·85 mm. in length.

A number of the filaments containing cells which had been unable to divide on account of the lack of potassium were carefully removed at 5 p.m., and placed in a watch-glass which contained some of their culture solution. During the night the temperature ranged from 15° to 18° C. When examined on the following day at 9 a.m. they were found to retain their former undivided condition.

In another experiment I tried the effect of transferring filaments from a culture in which sodium had been substituted for potassium, and in which the cells were consequently unable to divide, to a culture solution in which ammonium salts had been substituted for potassium. This transfer from

one solution to another was made at the time the cultures were taken from the refrigerator, and just before submitting them to optimum conditions. Seven and one-half hours later, I found upon examination that a few cells had undergone division. Evidently there had been sufficient stimulus in merely transferring the cells to a different solution to induce a few cases of division, although no potassium salts had been given by the transfer. Whether the stimulus was of a physical or chemical nature remains to be determined.

The effect of adding a salt of potassium to cultures in which the cells were unable to divide in consequence of a previous lack of potassium was noted upon some of the material just described. At the time of removing the material from the refrigerator, a few drops of a 2 per cent. solution of potassium phosphate were added to one of the cultures. Seven and one-half hours later I found that there had been some cell-divisions accomplished, but that many (perhaps a majority) of the cells had remained undivided, although they plainly showed the effect of the stimulus. This may have been due to one of two reasons. Potassium might have been necessary for certain of the initial processes and, lacking that, the whole mechanism of mitosis came to rest. Again, the failure to divide may have been due to lack of surplus nucleo-proteid material, and in the time of the experiment the cells were unable to use the new supply of potassium to form the requisite amount of nucleo-proteid.

2. Experiments upon the rôle of phosphorus.

A suitable supply of phosphorus is of fundamental importance for the continued activity of every living cell. Growth does not occur in the absence of phosphorus, because it is of primary importance for the formation of proteids. Koch and the writer have shown (Koch and Reed, '07) that when only an insufficient amount of phosphorus is present, the phosphorus of the nucleo-proteids shows practically no percentage decrease, while the water-soluble forms of organic and inorganic phosphorus show a relatively great decrease. The same conclusion may be drawn from the extensive analyses recently published by Wilfarth, Römer, and Wimmer ('05). They have shown that the amount of P_2O_5 in barley straw per hectare decreased from 29·04 kilograms on June 17 to 9·59 kilograms on July 27. The amount of P_2O_5 in the grain per hectare increased from 3·54 kilograms on June 17 to 29·84 kilograms on July 27. These results indicate that the nuclein phosphorus of grains increases in amount at the expense of the water-soluble forms of phosphorus in the straw.

Phosphorus is also necessary for the formation of the glycero-phosphoric acids, of which lecithin is one of the best known, and which is present in nearly every living cell. The physiological importance of lecithin for plants has been pointed out by Loew ('99), Overton ('00), and others.

Overton has concluded that lecithin plays an important rôle in the absorptive properties of the cell. He thinks that only those substances are absorbed which are soluble in the oily or fatty substances such as lecithin and cholesterin, which impregnate the cell-wall. Pfeffer remarks in this connexion that such impregnation may play an important part in regulating absorption, but, at the same time, the proteid constituents of the plasmatic membrane are also of importance. Lecithin is soluble to some extent in water, and may serve as a vehicle for the assimilation of fats. It is rather improbable that the chief function of lecithin is to serve for respiration, as Loew suggested. It may be pointed out that those tissues of animals which contain relatively large amounts of lecithin are not necessarily active agents in respiration.

Hydrodictyon died within two weeks after being transferred to a nutrient solution containing no phosphorus. The cells which died during the first few days of the experiment contained considerable starch, but where the cells died slowly the pyrenoids and their deposits of starch seemed to be consumed. Whether the cells died slowly because they were able to make use of the material of which the pyrenoids were composed, I was unable to determine.

When filaments of *Spirogyra* were cultivated in solutions which lacked phosphorus they showed the injurious effects at the end of three weeks. At that time many of the cells were dead, and the remainder showed evidence of greater or less injury. The contents of the dead cells were completely broken down; the nucleus having broken from its radiating strands of protoplasm was usually at the end or upon one of the lateral walls of the cell.

There appeared to be three well-marked stages in the death of *Spirogyra* cells in the solutions lacking phosphorus. In the first stage, the cell contents appeared cloudy, and the Marchi test usually showed that fats were present. This was undoubtedly due to the fact that the usual formation of lecithin was not going on, owing to the absence of phosphorus, and in consequence there was an accumulation of fats in the cells. In the second stage the chlorophyll bands became disarranged, either by taking a position nearly parallel to the long axis of the cell, or by rolling up in close spirals. In the third stage all green pigment disappeared from the chloroplasts, and the cell contents suffered complete disorganization. At the same time the filaments themselves were broken up in a very characteristic manner. Some time previous to the death of the filaments they had broken up into short rods composed of two or three cells, and, in many cases, the individual cells were entirely separated from neighbouring cells. The cells in this isolated condition appeared to suffer no more injury or to die no sooner than those which retained their normal relations.

In the material studied there was no evidence that the absence of phosphorus interfered with the process of starch formation. Cells which had died as a result of the absence of phosphorus displayed distinct rings of starch in the pyrenoids, and there were numerous starch grains in chloroplasts which were so seriously injured that their green pigment had disappeared. It seems hardly probable that all of this starch can be accounted for by assuming that it is identical with that present when the plants were transferred to the phosphorus-free solution. I have previously described how the starch originally present in these forms disappeared when they were transferred to solutions lacking potassium. It seems plausible to believe that a portion of this starch had been formed after the plants were put into the phosphorus-free solutions.

The transformation of starch into water-soluble carbohydrates was seriously impaired in cells deprived of phosphorus. In its absence starch was transformed into unusual forms of carbohydrate. Some cultures of *Spirogyra* in phosphorus-free solution were kept in diffuse light at rather low temperature for four months. This treatment tended to prolong the life of the cells by lowering their activity. When, at the expiration of that time, the filaments were examined microscopically they showed no signs of growth, although most of the cells did not seem to have been seriously injured. In nearly all cases there was a considerable quantity of starch remaining in the cells. When they were submitted to the action of potassium iodide iodine solution for twenty to thirty minutes they showed the presence of erythrodextrin. In order to make sure that the reacting substance was really erythrodextrin, I placed several filaments of the alga in a watch-glass containing a weak solution of diastase. At the end of two and one-half hours the tests were inconclusive, but at the expiration of eighteen hours there was hardly a trace of erythrodextrin remaining in the cells which had lain in the diastase solution.

The formation of cellulose was increased in a striking manner in the same plants in which erythrodextrin was demonstrated. The normal thickness of cell-walls in the control cultures of *Spirogyra* was 5 or 6 microns, but when grown in the absence of phosphorus many cells possessed walls 10 and 12 microns thick. I also observed thickened cell-walls in *Basidiobolus* which was cultivated upon phosphorus-free media. It would appear in these cases that it was not possible for starch to be fully hydrolyzed, but instead that it was merely transformed into dextrin, which probably represents one of the stages in the hydrolysis of starch. It was also possible for starch to be changed to cellulose, probably its polymeric form.

The course of the carbohydrates in the cells living in deficient nutrient solution indicates quite clearly that the lack of phosphorus interfered with the production or action of the enzymes which normally convert starch

into various saccharides. Loew and Bokorny ('87) reported that glucose occurs in demonstrable amounts in cells of *Spirogyra* during the process of conjugation, and also in cells which slowly perish in unfavourable conditions. It may be possible that these cells normally form glucose, and that its accumulation is due to the fact that the machinery of the cell is in no condition to make proper use of it.

The results of various experiments agree in showing the necessity of phosphorus for the production of proteids and proteid-like bodies. The injurious effect upon enzyme formation is, perhaps, analogous to the rapid disappearance of the water-soluble forms of phosphorus from the cell in cases of phosphorus-starvation observed by Koch and myself ('07). The diastase would probably be one of the first components of the cell to disappear, since, as I have shown in a former paper ('04), the production of enzymes is quickly influenced by external conditions.

The separation of the cells of the filaments from each other was probably brought about through conditions which dissolved the middle lamella of the cell-wall. The middle lamella, as first pointed out by Payen ('46), is mainly composed of calcium pectate, but in young unmodified cell-walls a considerable quantity of pectose is present, and a correspondingly smaller amount of calcium pectate. In the cell-walls under consideration, pectose undoubtedly forms a part of the middle lamella. Apparently the conditions brought about in the cell through the absence of phosphorus resulted in the formation of some substance in the cells (or in the culture-solutions), which dissolved the calcium pectate and pectose, and allowed the cells to separate. This question seems worthy of more extended study than I have been able to give it.

To study the rôle of phosphorus in germination I made several cultures of moss spores. Part of the cultures were made in the complete nutrient solution, and others in solutions containing all necessary elements except phosphorus. The spores were obtained from a species of *Atrichum*, and cultivated in solutions from March 9 to May 10. On the latter date the complete nutrient solutions contained large branched protonemata, which were developing buds and rhizoids. In the solutions lacking phosphorus there had been no germination of the spores.

With different solutions and different species of mosses, Schoene ('06) was able to germinate spores on phosphorus-free nutrient solutions. *Funaria hygrometrica* spores germinated, but produced only rhizoids in place of the normal protonema. *Bryum caespiticium* spores germinated on the deficient solutions, forming protonemata of an inferior size relative to the controls. The small protonemata grew for about ten days, and then began to turn brown at the apices. The injury would spread within a few days until the entire protonema was brown and all activity ceased.

The process of mitotic cell-division was studied in the absence of

phosphorus in the same manner as described in the experiments where potassium was absent.

A large culture of *Spirogyra*, which had grown for thirty-five days in the absence of phosphorus was subjected to a temperature of 5° C. during the night. At 9 the next morning it was placed in good light at a temperature of about 20° C. Two hours later it was examined with a microscope to ascertain what effect the lack of phosphorus would have upon the processes of nuclear and cell division.

It was evident from the first that the tendency to divide was manifested by fewer cells than in experiments where only potassium was absent. The filaments in the phosphorus-free solutions were in a living condition, yet a comparatively small number of cells showed any elongation or growth of the cells similar to that noted in cultures where potassium was absent. This may have been due either to a lack of suitable materials for growth, or, what is more likely, to the lack of simpler soluble substances, which by their reactions could have supplied the energy necessary for mitosis. The few cells which showed a tendency to divide had elongated to approximately twice their length. The nuclei also were attenuated in the direction of the longer axis of the cell, but there were no cases of division to be found, although at the same time there were a great many dividing cells in the control cultures. Three and one-half hours after taking the material from the low temperature, I divided the material in which no mitoses were occurring into two portions. One portion was kept unaltered for a control, to the other I added a few drops of a 2 per cent. solution of potassium phosphate. At the end of an hour the material was examined, and I found that the addition of the phosphorus had enabled some of the cells, which had been previously stimulated, to accomplish the process of division. The culture to which no potassium phosphate had been added showed no cases of mitotic divisions.

On the whole the experiments go to establish the predominating importance of phosphorus for the metabolism of the cell. When, for example, spores containing a small amount of reserve materials were put into a solution lacking phosphorus they were not able to germinate. When living cells were transferred to phosphorus-free solutions they soon ceased to grow. When cells succeed in living for a time in the phosphorus-free solutions their metabolism is impaired, and abnormal products are formed.

3. Experiments upon the rôle of calcium.

Most green plants, except certain of the Cyanophyceae, appear to require a suitable supply of calcium for normal growth. Relatively small amounts of calcium appear to suffice for the nutrition of most plants. It will not be safe to make such broad claims for the necessity of calcium in

plant growth, as some writers have done. Bruch ('02) has called attention to the fact that, aside from being unnecessary for Fungi and the lower Algae, it is quite toxic to certain swamp and aquatic plants.

The analysis of a plant usually shows that the greater part of the calcium is deposited in the stems and leaves, which at least suggests that it is concerned with the synthetic formation or transportation of organic products. Calcium forms a relatively small part of the seeds and fruits of most plants. The amount of calcium in diseased or etiolated leaves is less than in healthy leaves. Smith ('93), in his studies of peach 'yellows', gave the percentage of calcium in the ash of healthy leaves as 40·58, and in the diseased leaves as 23·88. In this connexion it may be proper to refer again to the results of von Portheim and Samec ('05). They found that in normal *Phaseolus* plants the amount of calcium was always greater than the magnesium, but that in etiolated plants the amount of calcium was often no greater than that of magnesium. All experimenters have observed that seedlings cultivated in solutions lacking calcium salts usually develop small leaves.

When I cultivated *Spirogyra* in a nutrient solution lacking calcium the chlorophyll soon showed signs of injury similar to that described by Bokorny ('95) and Loew ('99). The lobate margins were lost, and the broad bands became narrowed to a mere line. During the first stages of injury the pyrenoids contained some starch, but as the chloroplast sustained further injury the starch was less distinct. After seven weeks the chloroplasts became vacuolated and disorganized.

The chloroplasts of *Zygnema* likewise showed striking injuries when that alga was cultivated in a calcium-free solution. The delicate star-like form of the chloroplasts was lost because the radiating points were withdrawn. In cells which showed the most injury from lack of calcium the chloroplasts were contracted into irregular masses which ultimately became vacuolated. The application of iodine solution showed that each chloroplast contained considerable starch even when the injury was quite severe.

After *Spirogyra* had been deprived of calcium for two months only the smaller part of the cells remained alive, and they showed marked indications of injury. I noted in such cases that the apical cell of a filament was generally the first, and often the only one to show evidences of injury. In addition to the injury to the chloroplasts, already described, I found that these cells were partly filled with a dark substance which rendered them more or less turbid. The accumulation of this turbid material was usually greatest in the vicinity of the nucleus. Marchi's test showed that the substance causing turbidity was lecithin or some body closely related to the lecithins.

In the majority of cases I failed to obtain much evidence which bore

directly on the antagonistic action of calcium and magnesium which was discovered by Boehm ('75) and investigated thoroughly by Loew ('92). In my study of the rôle of calcium in Vaucheria, some evidence was obtained upon this question which is conclusive enough, so far as it goes, to warrant mention in this place. On April 5 a series of cultures of this alga was made in nutrient solutions lacking calcium and also in 0·1 per cent. solutions of calcium nitrate and of magnesium sulphate. The substitution for calcium salts in the formula of Beijerinck was made in two ways. In the first, magnesium chloride was substituted for calcium chloride, thus increasing the amount of magnesium about 50 per cent., and at the same time removing the antidoting agent, calcium. In the second, sodium chloride was substituted for calcium chloride. This solution may be considered as being quite different physiologically from the first because sodium, as was shown by the work of Kearney and Cameron ('02), acts as an antidote for magnesium. The filaments selected for these cultures were in a healthy growing condition, and contained a copious amount of oil droplets. In a few cases there were incipient zoosporangia at the tips of the filaments. At the end of eleven days there were evidences of injury to the plants in the solutions lacking calcium salts. The injury was found to be far greater in those solutions in which magnesium had been subsituted for calcium. There, many of the filaments were dead and broken up. The chloroplastids had lost their green pigment, and no oil droplets were to be found. The cultures in which sodium had been substituted for calcium contained material which was still living, although more or less injured. The chloroplastids were still green, and the protoplasm contained some oil droplets. In the control cultures there were numerous incipient oogonia and antheridia, but no signs of either were noted in the calcium-free solutions. I ultimately succeeded in keeping *Vaucheria* alive for seven weeks in cultures in which sodium had been substituted for calcium.

In the 0·1 per cent. solutions of calcium nitrate, the filaments were healthy and showed signs of growth by their numerous oil droplets and bright green colour. The incipient zoosporangia in these cultures had developed so far as to form the transverse walls separating the contents of the sporangium from the rest of the filament. The contents of the sporangia had, however, failed to develop zoospores. The filaments in this solution were developing sexual organs *pari passu* with the controls. In contrast, I found that all the filaments in the 0·1 per cent. solution of magnesium sulphate were dead.

It appears from these experiments that a large part of the injury was due to the toxic action of the magnesium, as well as to the absence of calcium. It seems reasonable to conclude that calcium is beneficial not only because of its value as a plant-food constituent, but on account of its power to antidote magnesium. This view is further supported by the

result of experiments in which sodium was added to solutions in which calcium was omitted, for there the lack of calcium seemed to be much less severely felt by the plants.

The spores of *Gymnogramme sulphurea* were able to germinate and produce normal prothalli in the absence of calcium salts. At the end of three months in the calcium-free solutions the prothalli were in good vegetative condition, and their meristematic cells contained a moderate amount of starch. The most striking variation from the normal course of development was found in the solutions where magnesium had been substituted for the missing calcium. The prothalli in these solutions had made very good growth, and had developed a very large number of antheridia, but no prothallus was ever found which had formed an archegonium. The cultures were continued for a total period of five months, during which time the prothalli in the control cultures developed both antheridia and archegonia, and, in course of time, young sporophytes. The prothalli in these calcium-free solutions did not, however, produce any archegonia. The antheridia which they produced were entirely normal, and produced normal antherozoids.

It will be noted that these results have a similarity to those obtained by Prantl ('81) who cultivated fern prothalli on solutions lacking nitrogen. On nitrogen-free solutions he obtained only antheridia, but on complete nutrient solutions both antheridia and archegonia. The development of archegonia on the prothalli appeared to be closely related to the formation of meristematic cells. In the cases which I have studied, the appearance of archegonia did not seem to be necessarily conditioned by the development of meristem.

It may be mentioned, in passing, that the nuclei of different sex cells of ferns differ, according to Zacharias ('87), in their chemical nature, and to this may be related the disappearance of one sort of cells under special conditions. The male sex cells are rich in nuclein and contain small nucleoli. The nuclei of the female sex cells are poor in nuclein, but rich in proteid and contain one or more nucleoli, often of large dimensions.

There is an obvious correlation between the structure of the nucleus and its activity, chemical or otherwise. In an earlier paper (Reed '04) I have shown that in the secreting cells of germinating seeds the nucleoli become smaller and the chromatin greater in amount as the activity of the cells increases. The observations there recorded indicate that the substance making up the chromatin and nucleoli is identical, the two forms differing mainly in their activity (or lability). Upon this hypothesis the nucleoli represent the less active or potential condition, while the chromatin represents the active or kinetic condition. Applying this theory to the case in hand, we may say that the active (kinetic) nuclei of the antherozoid-forming cells are able to obtain the materials from the incomplete solutions,

and, perhaps, from the neighbouring cells, necessary for their existence and growth. The more sluggish nuclei of the incipient female sex cells being unable to obtain the necessary substances for growth and activity, did not develop far enough to form sexually mature cells.

Some instructive facts upon the rôle of calcium salts were obtained by studying the process of cell-division in filaments of *Spirogyra* in calcium-free solutions. The conventional method of submitting the cultures to a low temperature during the night in order to stimulate mitotic division was adopted. Cultures of *Spirogyra* which had grown for six weeks without calcium salts showed evidences of some injury, yet the nuclei were able to divide. The mitosis was found to be in progress in many cells one hour after the cultures were removed from the refrigerator. Three hours and forty minutes after removal from the refrigerator I found many instances of mitosis, some of which were approaching completion. So far as the substance of the nuclei themselves was concerned, the mitotic divisions appeared to be typical, but the new transverse cell-wall was frequently incomplete, and still more frequently was entirely lacking. The appearance of the cells closely resembled those described by Molisch ('95) under similar conditions. In the control cultures the mitoses were normal and had formed perfect cell-walls between the daughter nuclei.

In the growing region of the root, a similar effect upon the process of mitosis was noted. Stained sections of the roots of *Zea Mais* plants, which had grown in calcium-free solutions, showed a number of cases in which the transverse septum had not formed after the process of nuclear division was completed.

A similar incapacity to form cellulose was found in the case of an alga which was about to form the thick walls of the resting spore. A quantity of *Spirogyra* was in the initial stages of conjugation when put into cultures on June 30. On July 13, when I examined the cultures I found that many of the filaments in the calcium-free solutions had succeeded in forming normal conjugating tubes, and that gametes had been produced. The most striking feature that I found was the case of conjugating cells which had fused to form a resting spore, but no cellulose wall was formed upon the surface of the plasmatic mass. In other respects the process of conjugation appeared to be normal, and I cannot explain the lack of cellulose forming-power unless it be caused by the lack of calcium salts.

The absence of calcium has been observed by several investigators to have a similar effect upon the power of the cell to form cellulose. It seems possible that some light might be shed upon the question by a consideration of the nature of the framework upon which the cell-wall is built. The middle lamella, as was first shown by Payen ('46), is composed of calcium pectate. Timberlake ('01) showed that the middle layer which appears after the splitting of the cell-plate in the later stages of mitosis may

be considered as the initial middle lamella. This middle layer appears to be formed originally by deposition from the split halves of the cell-plate, which were in turn derived from thickenings on the kinoplasmic spindle fibres. It cannot be assumed, however, that the middle lamella consists only of the material first deposited from the young plasma membranes. We have evidence rather that it consists of the material first deposited by the plasma membranes plus a certain amount of material subsequently deposited in contact with the layers. The writer believes that the absence of new cell-walls after mitosis, in cells lacking calcium, indicates that the cells were unable to form the fundamental 'middle layer' of the cell-plate, and, in consequence, no cell was formed.

It appears from what has been said that the rôle of calcium differs in certain respects from that of the elements previously described. It differs quite widely from potassium and phosphorus in forming but a small proportion of the actual living substance of the plant. The functions which it performs are varied, but are none the less specific on that account, and its absence may affect some of the most vital activities of the plant. Calcium influences, in a remarkable way, processes and products into which it does not seem itself to enter, although it may be necessary for the formation of the zymotic and other agents which accomplish the results. It is now recognized that calcium salts act beneficially upon the soil mainly through their ability to bring about the proper physical and chemical conditions for growth without serving directly as a nutrient. It is entirely possible that it may so function inside of the plant in the living substratum.

A conspicuous example of how this function may be performed is in the formation of calcium oxalate. By precipitating the poisonous oxalic acid in an insoluble form, the juices of the plant are maintained in a proper condition of acidity for their most effective work. It was shown, for example, by Groom ('96) that diastase was unable to accomplish the normal conversion of starch to sugars in the presence of oxalic acid.

It seems probable from such work as that of Wehmer ('06) that the plant is not altogether able to regulate the production of oxalic acid, as Pfeffer believes. It is, however, undoubtedly able to regulate to some extent the amount of calcium absorbed, and hence to control in that way the processes into which calcium enters. It is altogether probable that the amount of calcium absorbed by the plant at different times would be found to be subject to wide variation.

The accumulation of lecithins and fatty substances, which I have found in the cell when calcium salts are lacking, is another example of an impaired activity in which we must believe that calcium only functions in a very indirect manner. The absence of egg cells and archegonia of the fern prothalli, and the inability of cells to form cellulose are other examples.

4. Experiments upon the rôle of magnesium.

Magnesium is needed for the complete development of all plants. It is relatively more abundant in the fruits and seeds than in stems and leaves. A part of the functions of magnesium appear to be accomplished directly, and a part are indirectly performed, as in the case of calcium.

Magnesium has been assumed by Loew ('92) to play an important part in the assimilation of phosphorus as phosphoric acid. I have found that the amount of phosphorus present influences the functioning of the magnesium and vice versa.

Since the spores of the mould fungi are quite rich in lecithins and oils, they afford a suitable object of experimentation in investigating such questions. Some experiments were conducted upon spore formation in *Aspergillus niger* with the purpose of obtaining some light upon this question. It was not possible to exclude entirely the magnesium or the phosphorus from these experiments, because an insufficient amount of mycelium would have been obtained. The formula for the nutrient solution was that used for cultivating *Basidiobolus* and described on a previous page. No agar agar was added since *Aspergillus* grows well upon the surface of a liquid medium. The amount of phosphorus was reduced to fractional parts of the normal ratio; the amount of magnesium was reduced in some experiments, and increased in others.

In the first experiment the phosphorus was reduced to one-fifth the normal amount, and the magnesium increased to nearly three times the normal, thus making the ratio of magnesium to phosphorus about fifteen times as great as in the control solution. The mycelium produced on 100 cc. of this modified solution was slightly better than that produced on the control, weighing 1·55 g. in comparison with 1.20 g. for the control. The amount of spore-formation was judged by the eye to be 10 per cent. of the control cultures. A second experiment was made to determine the effect of decreasing the amount of phosphorus and magnesium present, while keeping the ratio equal to that of the control culture. One-tenth the normal amount of each element was used. The mycelium which grew on this solution produced approximately 50 per cent. as many spores as the controls, and approximately five times as many as upon the solutions in which magnesium predominated. These results are interpreted to mean that the surplus of magnesium in the first experiment was unfavourable to the development of spores. The particular point which seems worthy of emphasis is that in the first experiment there was twice as much phosphorus present as in the second; yet, on account of the great predominance of magnesium, conditions were not so favourable for spore-formation as in the second experiment. In other words the maintenance

of a proper ratio between magnesium and phosphorus was of more importance than the absolute amount of phosphorus present.

The filaments of *Spirogyra* which were cultivated in solutions lacking magnesium continued to live for some time, but showed characteristic injury. At the end of five weeks I found the chlorophyll bands disarranged and forming a more or less compact mass near the centre of each cell. A typical cell is represented in Fig. 2. In the cells which showed less injury, the nuclei were imprisoned within the irregular mass of chloroplasts; but in the more severely injured cells the nuclei had often lost their protoplasmic fibrillae and could be found in various abnormal positions in the cells. The state of affairs would seem to indicate that the fine strands of protoplasm radiating from the nucleus to the chloroplasts had been acted upon by some agent which caused them to contract simultaneously. In

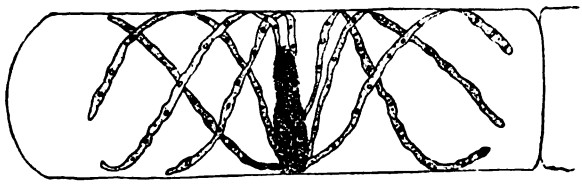


FIG. 2. *Spirogyra* cell grown six weeks in magnesium-free solution. Shows the contraction of the chloroplasts away from the ends of the cell. Drawn from a living cell mounted in water.

most cases the contraction had been violent enough to draw the chlorophyll bands away from the ends of the cell, and to make a more or less compact mass in the centre of the cell.

From a study of their behaviour it seems correct to ascribe the effects produced to the calcium present which is, in the deficient solutions, unbalanced by magnesium. Such an effect has at least an analogy, and perhaps more, in the effect of calcium on the muscle of animals. It has been shown by Greene ('99) and by Howell ('01) that strips of heart muscle will give a characteristic series of beats when placed in an isotonic solution of sodium chloride. A solution of calcium chloride, on the contrary, increases the tonus of the muscle which may pass into a state of permanent rigor from which recovery is impossible.

The chlorophyll bands in these cells were narrow, similar in many respects to those in cells deprived of calcium salts. The characteristic lobed margins were also lacking. In some cases the chloroplasts had been so sharply bent that they were broken at the centre, thus giving two groups of chloroplasts with a very narrow space between them. The pyrenoids stood out distinctly and appeared to be nearly, if not fully, normal size. There was an absence, however, of accessory pyrenoids (or dark staining bodies) similar to those appearing in the chloroplasts of the control plants.

By the use of stained preparations I was able to study the form of the nuclei in cells which showed signs of injury. I was unable to observe the shrunken nuclei which Bokorny ('95) observed under similar conditions. On the contrary the nuclei presented an unusually firm, even contour, because most of them had lost the enveloping layer of protoplasm with its radiating strands.

On account of the peculiar effect which the absence of calcium had upon the development of sexual organs on the prothalli of *Gymnogramme sulphureum*, it may be of interest to note the effect of the absence of magnesium. The spores of that fern when sown upon the surface of a nutrient solution germinated and grew very well. At the expiration of three months there were large numbers of well-developed prothalli on these solutions. A considerable quantity of starch was present in the younger cells on the lateral lobes, but less was present in the cells near the apical notch. Archegonia were quite numerous upon the ventral surface of the prothalli, several archegonia being found near the apical notch on almost every prothallus examined. The small amount of magnesium originally present in the spore of the fern was evidently sufficient for quite extensive growth in this case.

The cultivation of *Vaucheria* in deficient nutrient solutions is, generally speaking, rather difficult. Many of my attempts met only with failure, but in the case of magnesium-free solutions, I met with somewhat better success. Material was used which had grown for two months in the laboratory. The filaments appeared to be in good growing condition, containing both chloroplasts and oil drops throughout. A few of the filaments were beginning to form oogonia, and judging from their form the species seemed to be *V. gemmata*. The cultures stood upon the sill of a west window, at room temperature from January 17 to February 7. Upon the last-named date I found that the filaments in the magnesium-free solutions were entirely without oil globules, although oil globules were abundant in the filaments in the control solutions. Otherwise the filaments in the magnesium-free solutions appeared perfectly healthy and not injured. Filaments taken from the same source were growing in solutions lacking calcium salts without showing any diminution in the number or size of the oil globules they contained. It seems that there is no escape from the conclusion that there is an intimate relationship between the presence of magnesium and the formation of vegetable oils. Such a relation was suggested by Loew ('99), and he called attention to the fact that analyses show that oil-containing seeds like those of flax and cotton contain much more magnesium than the starch-containing seeds, like those of the cereals. Aso ('01) has published analyses of the spores of *Aspergillus oryzae* which show that those spores contain a moderately large amount of magnesium. This fact seems of importance because we know that the spores of the Fungi

store most of their reserve food in the form of oil. Still more important is the work of Sullivan ('05) who showed that, in order to form lipochromes to advantage, certain bacteria required the presence of magnesium sulphate and a phosphate, preferably potassium phosphate. The presence of relatively large amounts of magnesium in oil-forming seeds and spores confirms what was said on a previous page regarding the necessity of magnesium for the assimilation of phosphorus. This necessity is the more evident when we remember that the formation of fat is usually preceded or accompanied by the formation of lecithin.

The inability of *Vaucheria* cells to form oils in the absence of magnesium salts seems to prove that the foregoing hypothesis is the correct one. It seems possible that this function of magnesium may be its most important one. Probably all cells require at least a small amount of fatty material for the performance of all their functions. In the formation of these fats magnesium undoubtedly plays an important part.

Spirogyra filaments which had grown for five weeks in the absence of magnesium were submitted to low temperature in the same manner as other cultures to induce cell and nuclear division. The nuclei of these cells appeared to respond to the stimulus more slowly than any of the others had done. In the cells which showed greatest injury no response whatever was shown. Three hours and forty minutes after being placed in optimum conditions some cells and nuclei showed the phase of elongation presaging division. It was nine hours after removing the cultures from the refrigerator that I found the first cases of actual division. The divisions appeared to be normal in every way, and left no doubt in my mind that the nuclei of *Spirogyra* are able to divide mitotically in the absence of magnesium salts.

V. Discussion of Results.

Before proceeding further it may be in place to discuss the nature of the conclusions which may be legitimately drawn from experiments like those related in the foregoing pages. Since many of the methods which I have employed are capable of quite general application, it seems probable from the results obtained that they can be successfully applied to the study of certain types of physiological questions.

Microchemical methods are observational in character, and, like most purely observational methods, can yield only qualitative results. When possible, they should be followed by quantitative work. This does not mean that qualitative methods of study are of less value; on the contrary, qualitative methods, being observational in character, are of the greatest value in all biological work, and will probably continue so for some time. A valuable feature of microchemical methods in physiological

research is that they enable the observer to localize processes and products of cell metabolism. In consequence, one may expect to learn more precise facts concerning the function of the different organs of the cell under different conditions. In this respect the study of physiology is put upon a cytological basis. A trend in this direction may be expected in the future, just as morphology has become so largely a cytological study. As more methods are developed by means of which qualitative tests may be made upon the cell, the use of such observations will increase.

Reverting to what has been said concerning the specific rôle of the various elements studied, it may be pointed out that they possess common characteristics as well as specific differences.

The absence of no one essential element seems to be prohibitive of spore germination, as a general rule. Of course the growth of the sporelings was never as good in the incomplete solutions as in the complete solutions used as controls. In other words, the slight amounts of the essential elements in the spore stored as reserve food are sufficient to sustain growth for a time in an environment which lacks one of the essential elements. The resulting germination or growth in any such case is modified by characters apparently belonging to individual species.

The most easily recognizable functions of the essential elements, and the most capable of being understood, are those in which the element in question is directly utilized in some form in the synthetic formation of some organ or substance in the cell. In such processes it is usually not difficult to demonstrate that the element in question enters directly into some compound or organ of the cell. As an illustration of such a function, the rôle of phosphorus in the formation of lecithin may be given. Unless the radical of phosphoric acid be present, there is no way of uniting the glycero-fatty acids to the cholin group. Hence, in the absence of phosphates, fatty substances accumulate in the cell. The same is true of the reason for the necessity of phosphorus in the formation of nucleoproteids. All chemists are agreed that phosphorus holds an important position in the grouping of the elementary substances which make up the nucleins. The failure of the nuclei of *Spirogyra* to divide in solutions lacking phosphorus can therefore be readily understood, upon the grounds that a necessary element in the formation of nucleoproteids was lacking.

A second class of functions which it has been shown that the essential elements are capable of performing, are those in which the element does not itself enter into the composition of the end product of the reaction. In such cases the essential element may possibly be looked upon as a necessary catalyser, or as a dominating factor in bringing about proper conditions for the specific reaction to take place. It is probable that the greater part of the functions which any element performs in the plant belong to this class.

It has been shown, for example, that potassium is necessary for starch formation, and calcium for starch transportation; yet neither of these elements enters into the composition, either of starch, or of the compounds which arise as partial products in the formation of starch. Where phosphorus was lacking, there was an abnormal production of cellulose from starch, and in some cases erythrodextrin was formed from the starch. These effects may very probably have been due to the absence of the proper enzyme, which in normal cells converts starches to the various sugars. In turn the absence of the phosphorus may have been responsible for the non-formation of the requisite enzyme. In such an event, it shows that the influence of the element in question is an indirect one.

The fact that there is reason to believe that the individual elements may control the formation of certain catalytic agents, leads up to the question whether the individual elements may not themselves act as catalytic agents in certain processes. Such reactions have been more graphically worked out for the action of toxic bodies upon protoplasm than for the reaction of nutrient substances.

The results also give evidence to show that the value of an element, or of a compound, may consist, in part, in its ability to act as a carrier of certain ions or radicals. Magnesium appears to be necessary in some cases for the formation of oils. It is not probable that magnesium enters into the composition of the oils in any form. It acts rather as a carrier of some radical, probably that of phosphoric acid, which is necessary for the formation of lecithins. Later these lecithins may be split up with the formation of fats.

Another indirect function which some elements may perform may be designated as an 'antagonistic' or 'antidoting' function. Calcium and sodium, for example, appear to be of value in many cases because of their power to neutralize or overcome the otherwise harmful effect of magnesium. Concerning the real method by which the antidoting is accomplished we have little exact knowledge. Judging from the results which have been obtained, the beneficial action of these elements seems to be associated with biological processes, and the value of any antidoting agent will probably vary with different species of plants. It is not out of place, however, to note that an extended study of the reactions of the essential elements with the products of cellular metabolism might afford data of interest in this regard. It is known, for example, that calcium hydroxide will precipitate lecithin, but if sodium chloride be present, no precipitation will occur.

In the present paper no attempt has been made to study the value of the different essential elements or their compounds as sources of energy. A study of that question belongs more properly in the domain of chemistry than the present investigation has been carried. The value of the elements

in this connexion has been well pointed out by Wilfarth, Römer, and Wimmer ('05).

The character of the changes which are produced when one of the essential elements is lacking seems worthy of attention. In the experiments which have been described above, no case was found in which the absence of an essential element caused serious modification in the form or structure of the strictly *living* parts of the cell. Careful observations were made upon different plant cells with special reference to any modifications of the form of the nuclei, of the amount, form, or arrangement of the chromosomes. No changes could be discovered which indicated in any way that the lack of an element caused any modification of form or structure in the strictly living organs of the cell. My results appear to be quite comparable to those obtained by Klemm ('95) in a study of the disintegration of the cell organs following the action of strong physical and chemical stimuli. Klemm found that, although deep-seated changes were produced in the plasmatic organs of the cell, often no morphological evidence of the injury could be found until death was close at hand. The changes of form or structure which were brought about by the absence of one of the essential elements were always observed in the *non-living* parts of the cell, e. g. cell-wall, chlorophyll, starch, oil, &c. For this reason those cells were most profitable for study which contained the most highly differentiated non-living bodies. The cells of the root-tips of higher plants do not contain non-living elements other than the cell-wall, consequently the study of those cells was comparatively unfruitful. In cells like those of *Spirogyra* which are quite highly organized, but exhibit no physiological division of labour, the lack of an essential element is most noticeable in a microchemical study. It should not be concluded from these statements, that the lack of an essential element has no effect upon the seats of the vital processes. In this connexion it is proper to call attention to the teratological phenomena observed by Molliard and Coupin ('03). These investigators found that when *Sterigmatocystis* (*Aspergillus*) *nigra* was grown in the absence of potassium, the normal form of conidial apparatus disappeared. In place of the normal conidiophores, variable outgrowths appeared.

On the other hand, it is more than probable that the modifications of the non-living structures of the cell are the expressions of more deeply-seated changes in the activities of the living mechanism. When at length the absence of an essential element becomes serious enough to stop or reverse the normal course of activity, all activity ceases, i. e. death ensues.

VI. Summary.

1. The algae studied thrive best in artificial cultures which remain neutral or slightly acid in reaction. The phanerogams studied thrive better in a solution which remains neutral or slightly alkaline in reaction.

2. Potassium salts were found necessary for the germination and growth of certain mosses. When a certain sub-minimal amount of potassium was present, there was some evidence that certain moss spores could germinate and avail themselves of sodium during the embryonic stages of development.

3. In all green plants studied, potassium was essential for starch formation.

4. Mitotic cell division did not take place without a suitable supply of potassium, although a certain amount of growth by stretching was possible. The transfer of stimulated cells from one potassium-free solution to another potassium-free solution of slightly different composition enabled a few cells to divide.

5. The lack of phosphorus seemed to be more injurious to the cells studied than the lack of any other element.

6. Cells in a state of phosphorus starvation first lose the soluble phosphorus complexes; later the injury extends to the strictly living organs of the cell, finally resulting in its death.

7. Phosphorus appears to be more closely connected with carbohydrate transformation than with the origin of those substances. In the absence of phosphorus abnormal transformations occurred.

8. In the absence of phosphorus no mitotic divisions were possible. Cells which had lived for some time in the absence of phosphorus were difficult to stimulate to divide.

9. Calcium appears to be necessary for the activity and growth of the chlorophyll and chlorophyll-containing organs.

10. One of the most important functions of calcium seems to be the antidoting power it possesses for overcoming the bad effects of magnesium. There is evidence that the same function may be performed in part by sodium.

11. Antheridia were produced in large numbers on *Gymnogramme* prothalli cultivated in solutions lacking calcium, but archegonia were not found.

12. Cell nuclei were able to divide mitotically in the absence of calcium salts, but new cell-walls were imperfectly formed, if at all. The inability to form cellulose was also exhibited by the zygotes of *Spirogyra*.

13. Spore formation in *Aspergillus niger* was more abundant when

magnesium and phosphorus were present in the ratio of the normal culture solution. An excess of magnesium over phosphorus was detrimental to spore formation.

14. Magnesium salts were necessary for the continued health and activity of the chloroplasts.

15. Oil was not formed in *Vaucheria* when grown in the absence of magnesium.

16. The nuclei of *Spirogyra* were able to divide mitotically when no magnesium salts were furnished, although the process was somewhat retarded.

17. The essential elements studied appear to function in two ways:—(*a*) as component parts of cell structures or fluids; and (*b*) as indirect agents in causing less understood physical or chemical conditions necessary for the proper functioning of the cell, whether as carriers of other ions, or as specific antidoting agents.

18. The non-living parts of the cell, e. g. cell-wall, starch granules, or oil globules, are the only ones visibly modified by the lack of an essential element. The strictly living portions of the cell did not manifest any morphological changes until they were killed.

BIBLIOGRAPHY.

ARBER, E. A. N. ('01 A): On the effect of salts on the assimilation of carbon dioxide in *Ulva latissima*. Ann. Bot., xv, 39, 1901.

——— ('01 B): The effect of nitrates on the carbon assimilation of marine algae. Ann. Bot., xv, 669, 1901.

ASO, K. ('01): The chemical composition of the spores of *Aspergillus oryzae*. Bull. Coll. Agr., Tokyo, iv, 81, 1901.

BECQUEREL, P. ('04): Sur la germination des spores d'*Atrichum undulatum* et d'*Hypnum velutinum* et sur la nutrition de leurs protonémas dans des milieux stérilisés. Compt. rend. acad. sci., cxxxix, 745, 1904.

BENECKE, W. ('07): Untersuchungen über den Bedarf der Bakterien an Mineralstoffen. Bot. Zeit., lxv, 1, 1907.

BERTHELOT and ANDRÉ ('88): Sur le phosphore et l'acide phosphorique dans la végétation. Compt. rend. acad. sci., cvi, 711–716, 1888.

BIRNER and LUCANUS ('66): Wasserkulturversuche mit Hafer. Landw. Versuchsst., viii, 128–177, 1866.

BOEHM, J. ('75): Ueber den vegetabilischen Nährwerth der Kalksalze. Sitzber. d. Wiener Akad. d. Wiss., lxxi, 287–304, 1875.

BOKORNY, TH. ('95): Ueber den Einfluss des Calciums und Magnesiums auf die Ausbildung der Zellorgane. Bot. Centralbl., lxii, 1, 1895.

BOUILHAC, R. ('94): Influence de l'acide arsénique sur la végétation des algues. Compt. rend. acad. sci., cxix, 929, 1894.

BREAZEALE, J. F. ('05): Effect of the concentration of the nutrient solution upon wheat cultures. Science, xxii, 146, 1905.

——— ('06): The relation of sodium to potassium in soil and solution cultures. Jour. Amer. Chem. Soc., xxviii, 1013, 1906.

BRUCH, P. ('02): Zur physiologischen Bedeutung des Kalziums in der Pflanze. Landw. Jahrb., xxx, 127–144, 1902.

CLARK, J. F. ('01): On the toxic value of mercuric chloride and its double salts. Jour. Phys. Chem., v, 289, 1901.

DASSONVILLE, CH. ('98): Influence des sels minéraux sur la forme et la structure des végétaux. Rev. Gén. Bot., x, pp. 15, 59, 102, 161, 193, 238, 289, 335, 1898.

DAVY, SIR HUMPHREY ('13): Elements of Agricultural Chemistry. Philadelphia (published in), 1821, p. 304.

DORSET, M. ('03): The desirability of phosphates as an addition to culture media for tubercle bacilli. 20th Ann. Rep. Bureau of Animal Industry, U.S. Dept. Agr., 1903.

DUGGAR, B. M. ('06): The relation of certain marine algae to various salt solutions. Trans. St. Louis Acad. Sci., xvi, 473–489, 1906.

GAUNERSDORFER ('87): Das Verhalten der Pflanze bei Vergiftungen speziell durch Lithiumsalze. Landw. Versuchsst., xxxiv, 171–206, 1887.

GRAFE, V. and PORTHEIM, L. v. ('06): Untersuchungen über die Rolle des Kalkes in der Pflanze. Anz. k. k. Akad. Wiss. Wien, math.-nat. Kl., xviii, 324, 1906.

GREENE, C. W. ('98): On the relation of the inorganic salts of the blood to the automatic activity of a strip of ventricular muscle. Am. Jour. Physiol. ii, 82, 1898.

GROOM, P. ('96): Preliminary note on the relation between calcium and the conduction of carbohydrates in plants. Ann. Bot., x, 91–96, 1896.

HALLIBURTON and MOTT ('02): The chemistry of nerve degeneration. Phil. Trans. Roy. Soc., London, B cxciv, 437, 1902.

HAMMARSTEN, O. ('96): Ueber die Bedeutung der löslichen Kalksalze für die Faserstoffgerinnung. Zeitsch. f. physiol. Chemie, xxii, 333–395, 1896.

HASELHOFF, E. ('98): Versuche über den Ersatz des Kalkes durch Strontian bei der Pflanzenernährung. Landw. Jahrb., xxii, 851, 1893.

HELLRIEGEL, H. ('67): Ann. d. landw. Wochenbl., vii, 299, 1867.

HELLRIEGEL, WILFARTH, RÖMER, and WIMMER ('98): Vegetationsversuche über den Kalibedarf einiger Pflanzen. Arbeiten deutsch. landw. Gesell., Heft xxxiv, 1898.

HOLZNER, G. ('67): Ueber die physiologische Bedeutung des oxalsauren Kalkes. Flora, l, 497, 1867.

HOWELL, W. H. ('01): An analysis of the influence of the Sodium, Potassium, and Calcium salts of the blood on the automatic contractions of heart muscle. Am. Jour. Physiol., vi, 181, 1901.

KEARNEY and CAMERON ('02): Some mutual relations between alkali soils and vegetation. Report 71, U.S. Dept. Agr., 1902.

KLEBS, G. ('96): Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen. Jena, 1896.

KLEMM, P. ('95): Desorganisationserscheinungen der Zelle. Jahrb. wiss. Bot., xxviii, 627, 1895.

KNOP, W. ('81): Untersuchungen über die Ernährung der Pflanze. Blomeyer's Ber. v. Landw. Inst. zu Leipzig, 1881, pp. 31, 51. Abstr. in Biedermann's Centralbl. f. Agrikulturchem., x. 322, 1881.

KOCH, W. and REED, H. S. ('07): The relation of extractive to protein phosphorus in *Aspergillus niger*. Jour. Biol. Chem., iii, 49, 1907.

KOHN, E. ('05): Zur Biologie der Wasserbakterien. Centralbl. f. Bakt., Abt. 2, xv, 690, 717, 1905.

KRAUS, G. ('97): Ueber das Verhalten des Kalkoxalats beim Wachsen der Organe. Flora, lxxxiii, 54, 1897.

KRÖNIG, B. and PAUL, TH. ('97): Die chemischen Grundlagen der Lehre von der Giftwirkung und Desinfection. Zeitsch. f. Hygiene u. Infect., xxv, 1, 1897.

LIVINGSTON, B. E. ('07): Further studies on the properties of unproductive soils. Bull. 36, Bur. Soils U. S. Dept. Agr., 1907.

LOEB, J. ('06): The stimulating and inhibitory effects of magnesium and calcium upon the rhythmical contraction of a jelly-fish (Polyorchis). Jour. Biol. Chem., I, 427–436, 1906.

LOEW, O. ('91): Ueber die physiologischen Functionen der Phosphorsäure. Biol. Centralbl., xi, 273, 1891.

——— ('92): Ueber die physiologischen Functionen der Calcium und Magnesiumsalze im Pflanzenorganismus. Flora, lxxv, 368–394, 1892.

——— ('98): Ueber die physiologischen Functionen der Calciumsalze. Bot. Centralbl., lxxiv, 257, 1898.

LOEW, O. ('98 A): Zur Frage der Vertretbarkeit von Kaliumsalzen durch Rubidiumsalze bei niederen Pflanzen. Bot. Centralbl., lxxiv, 202, 1898.

——— ('99): The physiological rôle of mineral nutrients. Bull. 18, Div. Veg. Phys. and Path., U. S. Dept. Agr., 1899.

LOEW, O. and BOKORNY, Th. ('87): Chemisch-physiol. Studien über Algen. Journ. f. prakt. Chemie, xxxvi, 272, 1887.

——— ('89): Ueber das Verhalten von Pflanzenzellen zu stark verdünnter alkalischer Silberlösung. Bot. Centralbl., xxxix, 369, 1889.

LYON, E. P. ('04): A biological examination of distilled water. Biol. Bull., vi, 198, 1904.

MAYER, A. ('69): Untersuchung über d. Alkoholgärung, 1869, p. 44.

MOLLIARD and COUPIN ('08): Sur les formes tératologiques du *Sterigmatocystis nigra* privé de potassium. Compt. rend. acad. sci., cxxxvi, 1695, 1903.

MOLISCH, H. ('95): Die Ernährung der Algen, I. Abh. Sitzungsb. Akad. Wien, math.-nat. Klasse, civ, Abt. I, 783, 1895.

——— ('96): Die Ernährung der Algen, II. Abh. Sitzungsb. Akad. Wien., math-nat. Klasse, cv; Abt. I, 633, 1896.

MONNIER, A. ('05): Les matières minérales et la loi d'accroissements des végétaux. Thèse, Genève, 1905, pp. 37.

MOORE, G. T. ('03): Methods for growing pure cultures of algae. Jour. App. Microscopy, vi, 2309–2314, 1903.

NOBBE ('70): Ueber die organische Leistung des Kalium in der Pflanze. Landw. Versuchsst., xiii, 399, 1870.

OSTERHOUT, W. J. V. ('06): On the importance of physiologically balanced solutions for plants. Bot. Gaz., xlii, 127, 1906.

OVERTON ('00): Studien über die Aufnahme der Anilinfarben durch die lebende Zelle. Jahrb. wiss. Bot., xxxiv, 669, 1900.

PAYEN ('46): Recueil des savants étrangers, ix, 148, 1846.

PIGORINI, L. ('07): La diminuzione della tossicità del nitrato di argento trattato con tiosulfato sodico e l'azione della luce su questo fenomeno. Atti R. Accad. Lincei, Cl. sci. fis. nat. mat. (5), XVI, (1), 359, 1907.

VON PORTHEIM and SAMEC ('05): Ueber die Verbreitung der unentbehrlichen anorganischen Nährstoffe in den Keimlingen von *Phaseolus vulgaris*. Flora, xciv, 263, 1905.

PRANTL, K. ('81): Beobachtungen über die Ernährung d. Farnprothallien und der Vertheilung d. Sexualorgane. Bot. Zeit., 1881, p. 753.

RACIBORSKI, M. ('96): Ueber den Einfluss äusserer Bedingungen auf die Wachstumsweise des *Basidiobolus ranarum*. Flora, lxxxii, 107–132, 1896.

RAULIN, J. ('69): Études chimiques sur la végétation. Ann. d. sci. nat. (5), xi, 93, 1869.

v. RAUMER ('83): Calcium und Magnesium in der Pflanze. Landw. Versuchsst., xxix, 253–280, 1883.

v. RAUMER and KELLERMANN ('80): Ueber die Function des Calciums im Leben der Pflanze. Landw. Versuchsst., xxv, 25, 1880.

REED, H. S. ('04): A study of the enzyme secreting cells in the seedlings of *Zea Mais* and *Phoenix dactylifera*. Ann. Bot., xviii, 267, 1904.

SAUSSURE, THEODORE DE ('04): Recherches chimiques sur la végétation. Paris, 1804, pp. 327.

SCHIMPER, A. F. W. ('88): Ueber Kalkoxalatbildung in den Laubblättern. Bot. Zeit., xlvi, 65, 81, 97, 113, 129, 145, 1888.

——— ('90): Zur Frage der Assimilation der Mineralsalze durch die grüne Pflanze Flora, lxxiii, 207, 1890.

SCHOENE, K. ('06): Beiträge zur Kenntniss der Keimung der Laubmoossporen und zur Biologie der Laubmoosrhizoiden. Flora, xcvi, 276, 1906.

SCHREINER, O. and REED, H. S. ('07): Some factors influencing soil fertility. Bull. 40, Bur. Soils. U. S. Dept. Agr., 1907.

SESTINI, F. ('91): Esperimenti di vegetazione del frumento con sostituzione della glucina alla magnesia. Le Staz. Agrar. Ital., xx, 256, 1891. See Centralbl. f. Agrikulturchem., 1890, p. 464, and 1891, p. 558.

SMITH, E. F. ('98): Experiments with fertilizers for the prevention and cure of peach yellows. U. S. Dept. Agric. Div. Veg. Path. Bull. 4, 1893.

STOHMANN, F. ('62): Ueber einige Bedingungen der Vegetation der Pflanzen. Ann. d. Chem. u. Pharm., cxxi, 285–338, 1862.

SULLIVAN, M. X. ('05): Synthetic culture media and the biochemistry of bacterial pigments. Jour. Med. Research, xiv, 109–160, 1905.

SUZUKI, U. ('00): Can strontium and barium replace calcium in phanerogams? Bull. Coll. of Agr. Tokyo, iv, 69, 1900.

TIMBERLAKE, H. G. ('01): The development and function of the cell-plate in higher plants. Bot. Gaz., xxx, 73, 154, 1901.

TRUE, R. H. and GIES, W. J. ('08): On the physiological action of some of the heavy metals in mixed solutions. Bull. Torr. Bot. Club., xxx, 390, 1903.

VILLE, G. ('60): De l'importance comparée des agents de la production végétale. La soude peut-elle remplacer la potasse? Compt. rend. acad. sci., li, 437, 1860.

——— ('61): De l'importance comparée des agents de la production végétale. Des composés phosporés utiles pour la végétation et des composés phosporés qui ne le sont pas. Compt. rend. acad. sci., liii, 832, 1861.

WEHMER, C. ('91): Die Oxalabscheidung im Verlaufe der Sprossentwickelung von *Symphoricarpus racemosus*. Bot. Zeit., xlix, 149–194, 1891.

——— ('06): Die Bildung freier Oxalsäure durch *Aspergillus niger*. Ber. d. deutsch. Bot. Gesell., xxiv, 381-384, 1906.

WHEELER, H. J. ('05): Plant peculiarities as shown by the influence of Sodium salts. Bull. 104, Rhode Island Agr. Exp. Sta., 1905.

WILFARTH, RÖMER, and WIMMER ('05): Ueber die Nährstoffaufnahme der Pflanzen in verschiedenen Zeiten ihres Wachstums. Landw. Versuchsst., lxiii, 1–70, 1905.

WINOGRADZKY ('84): Ueber die Wirkung äusserer Einflüsse auf die Entwickelung von *Mycoderma vini*. Bot. Centralbl., xx, 165, 1884.

WLASSAK, R. ('98): Die Herkunft des Myelins. Archiv f. Entwickelungsmechanik, vi, 453, 1898.

WOLF, W. ('64): Die Saussure'schen Gesetze der Aufsaugung von einfachen Salzlösungen durch die Wurzeln der Pflanzen. Landw. Versuchsst., vi, 203–230, 1864.

WOLF, G. ('68): Bericht über die in den Jahren 1866 und 1867 ausgeführten Vegetationsversuche in wasseriger Lösung der Nährstoffe. Landw. Versuchsst., x, 349–379, 1868.

YERMAKOV, V. ('05): Correlation between calcium salts and the assimilation of nitrate nitrogen. Russ. Jour. Expt. Landw. vi, 403–431, 1905; Rev. in Exp. Sta. Rec. xvii, 1144, 1906.

ZACHARIAS, E. ('87): Beiträge zur Kenntniss des Zellkerns und der Sexualzellen. Bot. Zeit., xlv, 280, 1887.

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